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EDITED FOR

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VOL. XCV—NO. 1

Issued October 1, 1930

BALTIMORE, U. S. A.

1930

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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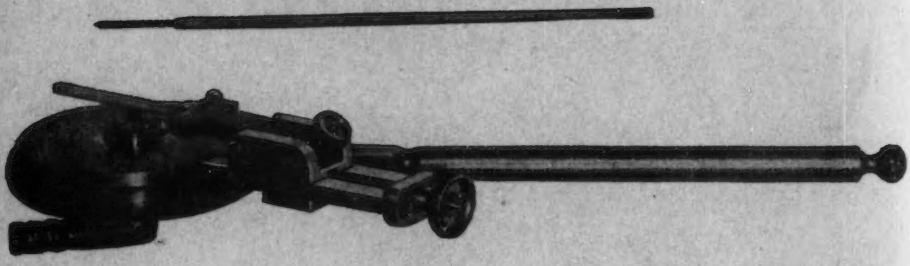

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 95 OCTOBER 1, 1930 No. 1

DO BLOOD PLATELETS, PLASMA, AND TISSUES YIELD THROMBIN, OR TISSUE FIBRINOGEN?

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Received for publication April 2, 1930

According to Bordet (1), thrombin is that substance obtained by the interaction, in the presence of calcium, of cytozyme (cephalin) of the platelets and tissues, and serozyme (prothrombin) from the blood plasma, the final product having the power to clot blood fibrinogen in plasma, or in its purified state, in the absence of ionic calcium. There are other substances capable of clotting fibrinogen in the absence of calcium, notably certain snake venoms and bacterial products (staphylococcus aureus, Gratia). Perhaps eventually they will be found to be identical with blood thrombin, but until then the term pseudo-thrombin or thrombinoid might be used for all such acting substances except that one derived from a blood source. Our studies on tissue fibrinogen action, expanding the earlier work of Wooldridge, necessitated a clear differentiation between this type of clotting and that by thrombin. Failure to keep in mind that ionic calcium is necessary for tissue fibrinogen action but not for thrombin, has led to confusing statements and conclusions. It is in the hope of clarifying this particular part of the blood clotting problem that this paper is offered.

In establishing Bordet's definition of thrombin, and in differentiating thrombin from tissue fibrinogen one question must first be answered: Is plasma the only source of serozyme (prothrombin), or do the platelets and tissues act similarly? The idea that the platelets were the source of thrombin was derived from certain observations: 1. Under the microscope the first fibrin was seen to form around the platelets, and from there to extend throughout the plasma. 2. Plasma deprived of its platelets clotted very slowly, while addition of excess platelets caused more rapid clotting and the production of a serum much richer in thrombin than that obtained from the plasma free of platelets. 3. Platelet suspensions, containing ionic calcium, would clot purified fibrinogen solutions, and therefore the addition of calcium to the platelets was thought to have caused

the formation of thrombin. The main error in the conclusion from such observations that thrombin arises from blood platelets is due to the failure to differentiate between thrombin action and that of the tissue fibrinogen. It is the tissue fibrinogen of the platelets, and not thrombin, that initiates this clotting. Likewise, the clotting of fibrinogen solutions by platelet suspensions, in the presence of calcium, is due to tissue fibrinogen, and not to thrombin, since it does not occur if the platelets are first exposed to calcium and thereafter oxalated and added to plasma or fibrinogen solution. These facts are brought out clearly by the data presented below.

Platelets were prepared from fresh citrated dog blood by fractional centrifugation, washing in 0.9 per cent NaCl solution, and finally shaking in distilled water. *Blood fibrinogen* was obtained from the platelet-free plasma by repeated salt precipitation and washing until the fibrinogen was so pure that it would not clot within 24 hours on addition of either cephalin or calcium, or both, but would clot promptly with thrombin or tissue fibrinogen and calcium. *Prothrombin* was obtained from the same plasma after removal of the fibrinogen by salting out methods as described elsewhere (2). *Cephalin* was freshly prepared from sheep brain and a 0.1 per cent emulsion made in 0.9 per cent NaCl solution.

The platelet solution was examined for thrombin by testing its ability to clot the purified fibrinogen solution without calcium addition:

0.5 cc. fibrinogen + 0.3 cc. platelet solution = *no clot in 24 hours.*

It was likewise tested for prothrombin by addition of cephalin and calcium, with subsequent oxalation and addition to the fibrinogen solution:

0.5 cc. platelet solution + 0.1 cc. cephalin + 0.1 cc. 1 per cent $CaCl_2$ (mixture kept at 20°C. for 5 minutes + 0.1 cc. 2 per cent potassium oxalate + 0.5 cc. fibrinogen sol. = *no clot in 24 hours (40°C.)*

A repetition of this experiment using the prothrombin solution in place of the platelet solution gave solid clotting of the fibrinogen in ten seconds.

Since the platelets, then, do not contain thrombin or prothrombin, let us next see whether they contain cephalin or tissue fibrinogen. First to show that both cephalin and calcium are needed for prothrombin activation:

0.3 cc. prothrombin + 0.5 cc. fibrinogen = no clot in 24 hours.

0.3 cc. prothrombin + 0.1 cc. $CaCl_2$ (mixture kept at 20°C. for 15 minutes) + 0.5 cc. fibrinogen = no clot in 24 hours.

0.3 cc. prothrombin + 0.1 cc. cephalin (mixture kept at 20°C. for 15 minutes) + 0.5 cc. fibrinogen = no clot in 24 hours.

0.3 cc. prothrombin + 0.1 cc. cephalin + 0.1 cc. $CaCl_2$ (mixture kept at 20°C. for 15 minutes) + 0.1 cc. 2 per cent potassium oxalate + 0.5 cc. fibrinogen = solid clot in 10 seconds.

We see that neither calcium nor cephalin alone can activate prothrombin, but that both together produce very active thrombin. Replacing the cephalin emulsion by the platelet solution, boiled or unboiled:

0.3 cc. prothrombin + 0.1 cc. platelet solution + 0.1 cc. $CaCl_2$ (mixture kept at 20°C. for 15 minutes) + 0.1 cc. 2 per cent potassium oxalate + 0.5 cc. fibrinogen = solid clot in 10 seconds.

0.3 cc. prothrombin + 0.1 cc. *boiled* platelet solution + 0.1 cc. $CaCl_2$ (mixture kept at 20°C. for 15 minutes) + 0.1 cc. 2 per cent potassium oxalate + 0.5 cc. fibrinogen = solid clot in 10 seconds.

These experiments make it very evident that the platelet solution, either boiled or unboiled, is capable of furnishing a copious supply of free cephalin for the activation of prothrombin.

That tissue fibrinogen is also present is evidenced by the ability of the platelet solution to clot the fibrinogen in the presence of calcium (but in the absence of prothrombin)

0.5 cc. fibrinogen + 0.2 cc. platelet solution + 0.05 cc. $CaCl_2$ = solid clot in 15 minutes.

0.5 cc. fibrinogen + 0.2 cc. *boiled* platelet solution + 0.05 cc. $CaCl_2$ = no clot in 24 hours.

0.5 cc. fibrinogen + 0.2 cc. 1 per cent tissue fibrinogen solution + 0.05 cc. $CaCl_2$ = solid clot in 6.5 minutes.

We may, therefore, say with certainty that the platelets do not serve as a source of either thrombin or prothrombin, but that their coagulant action is due to their tissue fibrinogen and cephalin content.

In seeking for thrombin or its precursor in tissue extracts, we used fresh rabbit tissues. Portions of lung, kidney, heart, liver, small intestine, spleen, bone marrow, skeletal muscle, testes, and thymus were washed as free of blood as possible, and then macerated in a mortar with ten parts of 0.9 per cent NaCl solution. To 1 cc. portions of these extracts were added 0.2 cc. portions of 0.5 per cent $CaCl_2$ solution. These calcified mixtures were kept at room temperature for 30 minutes to allow of activation of any prothrombin present. Since the tissue extracts have been shown to contain free cephalin, this calcium addition should be sufficient for thrombin production if prothrombin be present. At the end of 30 minutes' standing, 0.2 cc. of each calcified extract was added to 0.5 cc. citrated beef plasma at 40°C. It had been previously determined that the amount of calcium contained in each 0.2 cc. of calcified tissue extract was insufficient to produce clotting of the 0.5 cc. of citrated plasma, but that the calcium in 0.5 cc. was sufficient for this purpose. *The 0.2 cc. addition produced clotting of the citrated plasma only in the case of the spleen extract, and then only after several hours.* Perfusion of the spleen for a few minutes with 0.9 per cent NaCl solution, followed by maceration and extrac-

tion, yielded an extract which would not clot the citrated plasma under the above conditions. This shows that the action of the spleen extract was probably due to thrombin derived from blood not entirely removed. These experiments show, then, *that no thrombin can be produced from fresh tissue extracts* by addition of calcium. Both calcium and cephalin were added to another series of tissue extracts followed by oxalate addition 30 minutes later; but these mixtures were also unable to clot citrated plasma.

To show that these calcified extracts do possess an active coagulant capable of quickly clotting plasma, in the presence of ionic calcium, 0.5 cc. of each was added to 0.5 cc. portions of citrated plasma and the clotting times noted.

CIT. PLAS.	CALCIFIED TISSUE EXTRACT	CLOTTING TIME
0.5	0.5 cc. Testes extract	20 seconds
0.5	0.5 cc. Liver extract	45 seconds
0.5	0.5 cc. Kidney extract	95 seconds
0.5	0.5 cc. Intestine extract	25 seconds
0.5	0.5 cc. Bone marrow extract	No clot
0.5	0.5 cc. Heart muscle extract	25 seconds
0.5	0.5 cc. Skeletal muscle extract	25 seconds
0.5	0.5 cc. Lung extract	25 seconds
0.5	0.5 cc. Thymus extract	30 seconds
0.5	0.5 cc. Spleen extract	30 seconds
0.5	0.1 cc. 0.5% $CaCl_2$ sol. (control)	10 minutes

We observe here very rapid clotting when enough of each extract is added for the calcium to more than neutralize the citrate present in the plasma. This coagulant action of the extracts is due to their content of tissue fibrinogen and free cephalin. The inhibitory action of the bone marrow extract, as seen here, is being studied more in detail.

Having failed to find prothrombin or thrombin in tissue extracts or blood platelets, it was sought in the plasma. Goose plasma was obtained without admixture of tissue juice by carefully inserting a paraffined cannula into the carotid artery of a fasting goose, under local anesthesia. The whole blood, without addition of oxalate or citrate, was drawn into chilled paraffin-coated centrifuge tubes, and centrifuged until entirely free of formed elements. This clear plasma was kept at room temperature in paraffined vessels for two weeks without showing any evidence of coagulation. Purified fibrinogen and prothrombin fractions were prepared from portions of it by salting out, reprecipitation, and washing. The activation of the prothrombin was tested as follows:

0.3 cc. goose prothrombin + 0.1 cc. cephalin + 0.1 cc. $CaCl_2$ (mixture kept at 20°C. for 15 minutes) + 0.1 cc. 2 per cent potassium oxalate + 0.5 cc. fibrinogen = solid clot in 40 seconds.

Evidently there can be no doubt as to the presence of an abundant supply of prothrombin in this unaltered plasma. This result corroborates the findings of Bordet and Delange. They removed prothrombin from the plasma by adsorption with tricalcium phosphate.

On other samples of the same goose plasma lipoid extractions yielded a phospholipin which was capable of assisting in the prothrombin activation. So plasma alone contains all the necessary thrombin generators: prothrombin, cephalin and calcium. Blood platelets and tissue extracts, on the other hand, contain no prothrombin or thrombin, but owe their coagulant action to free cephalin and tissue fibrinogen.

It is unfortunate that Loucks and Scott, in their coagulation work reported in a recent article (3), failed to differentiate between thrombin and tissue fibrinogen action. They claim to have demonstrated thrombin in calcified tissue extracts, thereby rendering unnecessary our idea of the two types of clotting. Their "tissue thrombin" clotting always took place in the presence of calcium, however, and was prevented by oxalation, so that it was tissue fibrinogen with which they were dealing. We have repeatedly attempted in various ways to derive thrombin from tissue fibrinogen, or tissue extracts, but entirely without success. We could find no preparation which would clot blood fibrinogen in the presence of excess oxalate, as thrombin will do.

These same authors also point out that the presence of excess oxalate produces an inactivation of blood thrombin. This inactivation, however, is *gradual* and not *instantaneous* as with tissue extracts or their "tissue thrombin."

We have verified their findings in regard to the effect of oxalate on serum thrombin. Serum, which might require hours of standing to reach a low level of thrombic activity spontaneously, is almost completely inactive 30 minutes after oxalation. The loss, however, is *gradual*. Calcified tissue extracts, on the other hand, keep a constant coagulative activity for many days, but become *instantly* inactive on oxalation. Furthermore active thrombin added to oxalated plasma will always induce rapid clotting, while calcified tissue extracts will do so only when there is more than sufficient calcium present to bind all the oxalate. We feel justified in asserting, then, that tissue extracts do not yield thrombin, but that their coagulative action is due to tissue fibrinogen, or cephalin.

In summary, then, it should be emphasized that a critical review of the pertinent facts at hand indicates:

1. That plasma alone contains, beside blood fibrinogen and calcium, prothrombin and cephalin.

2. That blood platelets do not yield thrombin or prothrombin, but do yield on extraction free cephalin and tissue fibrinogen.

3. That tissue juices likewise yield no extractable thrombin or prothrombin, but do yield free cephalin and tissue fibrinogen.

4. That there are at hand methods of properly identifying these various clotting agents, methods which should receive proper consideration in any attempted study of the clotting problem.

The author wishes to express his appreciation of the helpful criticism and suggestions of Dr. A. P. Mathews. His interest in the problem of blood clotting has been a most stimulating influence during the past eleven years of work.

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ACTION OF INSULIN ON THE MOTILITY OF THE GASTRO-INTESTINAL TRACT

VI. Antagonistic Action of Posterior Pituitary Lobe Preparations

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Received for publication June 9, 1930

It has repeatedly been shown that posterior pituitary lobe preparations will prevent or relieve many of the effects produced by insulin. They prevent the fall of blood sugar, the convulsions and depression which follow the administration of insulin if the two substances are given simultaneously, or abolish these effects if given after the insulin (Burn, 1923). It has not been established so definitely whether this antagonism applies to all the fractions of pituitrin for Houssay (1929) reported that oxytocin is much more effective than vasopressin, while Burn (1928) found that insulin was antagonized by vasopressin but not by oxytocin.

Insulin has been found to increase the motility of the empty stomach (Bulatao and Carlson, 1924) and of the small intestine and colon (Quigley and Solomon, 1930); this action is related to the development of hypoglycemia. A study of gastro-intestinal motility, therefore, offers an excellent method for investigating the antagonism between insulin and pituitary extract or its fractions.

Before it is possible fully to evaluate any antagonistic action on gastro-intestinal motility it is important to know the action of pituitary extracts on spontaneous motility. The extensive literature is contradictory but in general indicates that augmentation of the tone and motility is usually obtained (see review by Geiling, 1926).

We have employed the balloon method to make determinations of the action of pituitary on the spontaneous motility especially of the stomach but also simultaneously on the small intestine and colon of normal fasting dogs. Since Carlson, Boyd and Pearcy (1922) and Carlson (1930) have shown that stimulation of the vagus or hypogastric nerves will augment or depress motor activity of the gut, depending on its initial tone; and furthermore, since clinicians employ pituitary preparations to relieve atony of the gut, we have determined the action of this substance in cases of low as well as high spontaneous tone.

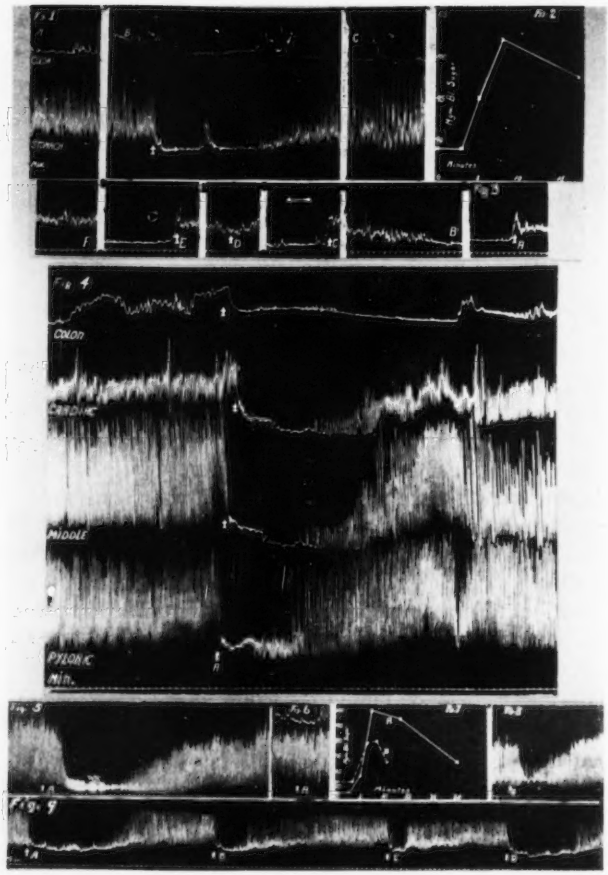

Fig. 1. Dog fasting 20 hours, spontaneous motility, *marked* activity. A, 36 minutes before pituitrin injection. B, intravenous injection of 0.4 cc. vasopressin-oxytocin. Gastric inhibition in 19 seconds. C, 93 minutes after injection.

Fig. 2. Blood sugar curve from B, figure 1. Injection made at 0 minute.

Fig. 3. Dog fasting 20 hours, spontaneous motility, *low* activity. A, intravenous injection of 0.4 cc. vasopressin-oxytocin. B, 25 minutes after A. C, 116 minutes after A. Intravenous injection of 0.4 cc. vasopressin-oxytocin. D, 78 minutes after C. Intravenous injection of 0.6 cc. vasopressin-oxytocin. D, 78 minutes after C. Intravenous injection of 0.6 cc. vasopressin-oxytocin exposed to 2 N NaOH at room temperature for 2 hours. E, 49 minutes after D. Intravenous injection of 0.4 cc. vasopressin-oxytocin 5 hours and 27 minutes after A. F, 40 minutes after E.

Fig. 4. Dog fasting 20 hours, balloon in colon and three balloons in stomach. A, 58 minutes after 20 units of insulin subcutaneously, injected intravenously 0.4 cc. vasopressin. Gastric depression in 18 seconds.

The balloon technique was also used to follow the hyperactivity of the gut produced by insulin administration and to determine the antagonistic action of pituitary extracts. Our thanks are due to Eli Lilly & Co. for the insulin of high purity.

Throughout our investigations the blood sugar level was frequently determined by the method of Somogyi (1926).

The employment of pituitary preparations which contained pharmacologically active impurities is responsible for many of the contradictions regarding the actions of pituitrin. We have made use of the highly purified pituitrin, oxytocin and vasopressin, kindly supplied by Parke, Davis & Co. and the infundin of Burroughs, Wellcome & Co. The pituitrin preparations were usually administered intravenously but intramuscular injections were found to yield similar results. Seventy-four experiments which gave definite results were performed and eleven dogs were used.

Action of pituitrin on the motility and blood sugar of normal animals. Pituitrin preparations invariably produced inhibition of the gastro-intestinal tract. There was never any indication of augmentation and there was also no indication that an injection given one hour after a previous injection was in any way modified by the previous injection (fig. 3). Pituitrin, oxytocin, vasopressin, vasopressin-oxytocin or infundin each gave results which were practically identical. Intravenous administration of 4 to 8 units (unit system of Kamm *et al*, 1928) apparently gave optimal effects, for the results were not more marked from doses twice as great. Smaller doses gave quantitative but not qualitative differences in effect (fig. 9). Administration of ten times the amount of preservative (chloretone) and acid found in the pituitary solutions did not modify the motility (fig. 6).

The characteristic effect of pituitrin preparations on the stomach was a very abrupt inhibition of tone and motility which became complete 15 to 20 seconds after the beginning of the injection. The loss of tone was usually maximal at this time but in some cases it was slightly greater at

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Fig. 5. Dog fasting 20 hours, 197 minutes after 20 units of insulin. A, intramuscular injection of 0.9 cc. oxytocin.

Fig. 6. Dog fasting 20 hours, 143 minutes after 20 units of insulin. A, intravenous injection of 4 cc. of an acidic solution of 0.5 per cent chloretone.

Fig. 7. A, blood sugar curve after A, figure 9. B, blood sugar curve after A of figure 8. Injections made at 0 minute.

Fig. 8. Dog fasting 68 hours, 128 minutes after injection of 20 units of insulin. A, intravenous injection of 1 cc. infundin exposed to NaOH for 75 minutes.

Fig. 9. Dog fasting 20 hours. A, 156 minutes after 20 units of insulin, intravenous injection of 0.4 cc. vasopressin-oxytocin. B, 39 minutes after A, intravenous injection of 0.2 cc. vasopressin-oxytocin. C, 36 minutes after B, intravenous injection of 0.1 cc. vasopressin-oxytocin. D, 25 minutes after C, intravenous injection of 0.4 cc. vasopressin-oxytocin.

the end of 2 or 3 minutes than after one minute. Recovery of tone usually began 15 minutes after the injection and was usually complete after 20 minutes. The inhibition of motility usually ran parallel to the loss of tone but in some cases the latter was more complete and the recovery of motility began occasionally while the tone was still very low.

Using the triple balloon in the stomach it was seen that the cardiac, mid and pyloric portions were depressed by the pituitary preparations in a similar manner (fig. 4). Depression was more abrupt in the pyloric end and usually recovery occurred earlier here but on other occasions motility returned first in the cardiac end or in the middle. Slight changes in the susceptibility of different parts of the stomach to pituitrin probably explain these variations.

The action of pituitary preparations on the motility of the small and the large intestine was similar to that on the stomach. A prolonged decrease in tone and motility was always obtained. Administration of pituitrin was found to produce inhibition in cases of low as well as high spontaneous tone of the gut (fig. 3).

Intravenous injections of pituitary preparations did not modify the blood sugar level during the 3 to 5 minute period following the injection (fig. 2). It then rose abruptly to reach a maximum at the end of the 7 to 10 minute interval. A definite tendency to fall was usually apparent after 14 minutes or less and a value approximating that noted prior to the injection was usually obtained by the end of 15 to 20 minutes. Values below this level were frequently seen at the end of 30 minutes.

It is thus evident that any of the pituitary preparations inhibit the gut in cases of high or low spontaneous motility and also produce a temporary elevation of the blood sugar level.

Antagonistic action of pituitrin and insulin. Pituitary preparations in doses of 4 to 8 units produced an inhibition of the gut in insulinized animals which closely resembled that noted in the normal dog. The inhibition in the former case was more striking but this was apparently due to the fact that the inhibition was complete both in the case of normal spontaneous activity and in the insulin hyperactive gut. All of the pituitary preparations had a similar effect on gastro-intestinal motility.

In animals to which insulin had been administered 1 to 4 hours previously the blood sugar values were usually about 42 mgm. per 100 cc. just preceding the administration of pituitrin. Each of the pituitary preparations appeared to produce the same change in the blood sugar curve. It closely resembled that obtained in the normal animal, but a fall was frequently observed during the first 3 minute period and the absolute rise following this interval was more marked in the insulinized animal (fig. 7).

Each of the pituitary preparations was usually found to be equally effective in relieving the depressive or convulsive effects of insulin. This relief

did not begin until 3 to 5 minutes after the intravenous injection of pituitrin and in general appeared to follow the blood sugar curve.

Mechanism of pituitrin inhibition. It has been shown by Bulatao and Carlson and by Quigley and Solomon that elevation of the blood sugar level, e.g., by injection of dextrose, frequently inhibits the motility of the gut in the normal or insulinized animal. Pituitrin produces an elevation of the blood sugar level and it was therefore possible that the inhibition was produced through this mechanism. However, this was made doubtful by the observation that the inhibition of the gut was abrupt in onset while the changes in blood sugar level displayed a pronounced latent period. It then became questionable whether the blood sugar level played any rôle in the inhibitory action of pituitrin. Information bearing on this problem was obtained by injecting infundin which had been exposed to 2N NaOH for varying time intervals. When the exposure was not greater than 30 minutes the typical effects of pituitrin on motility and blood sugar were obtained. If the exposure was extended to 60 or 75 minutes the response of the gut was modified in that inhibition did not begin until about 4 minutes after the injection but the blood sugar curve was practically typical (fig. 7). Pituitary preparations exposed to NaOH for 150 minutes were without effect on motility or blood sugar level. These effects were obtained on the normal and also the insulinized animal.

It is thus apparent that the inhibition of the gut produced by pituitrin involves two mechanisms. The first probably consists of a direct paralyzing action on the motor mechanism of the gut. This effect is of rapid onset and the substance producing the action is relatively labile to the destructive action of NaOH. The second mechanism is related to the elevation of the blood sugar and is due to a substance relatively stable towards NaOH. It is comparatively slow in acting. As the blood sugar returns to its former level, the inhibition of the gut disappears. Inactivation of the pituitrin has apparently occurred by this time.

It may be suggested that an elevation of the blood sugar level cannot be demonstrated during the first 3 to 5 minutes following the injection of pituitrin because the tissue cells are removing the liberated sugar so rapidly that it cannot be found in the venous blood. This appears unlikely for Hines, Jacobs and Leese (1928) found that pituitrin retards the removal of injected glucose from the blood and it has also been shown by Hines, Leese and Boyd (1927) and by Lambie and Redhead (1929) that pituitrin administration decreases carbohydrate metabolism. It appears more likely that pituitrin does not appreciably modify the blood sugar regulating mechanism during the first few minutes following administration.

Discussion. Pituitrin preparations inhibit the gut made hypermotile by the action of insulin. We do not consider this an example of *specific* antagonism of insulin by pituitrin in the sense that one destroys or neu-

tralizes the other or even that they act directly on the same tissue, for pituitrin inhibits spontaneous motility in an identical manner but there is practically no evidence to indicate that spontaneous motility is dependent on active insulin in the animal. Furthermore, optimal depression of the gut is obtained from the same dose of pituitrin in the normal and the insulinized animal. Pituitrin inhibition of the gut, like the relief of other indications of insulin over-dosage, is probably produced indirectly by overcoming the insulin hypoglycemia. Pituitrin inhibits the gut also by depressing the motor mechanism.

SUMMARY

Since pituitrin prevents or relieves many of the actions of insulin, information regarding this antagonism can be derived from a study of the action of pituitrin on the hypermotility of the gut produced by insulin. Pituitrin, vasopressin, oxytocin, vasopressin-oxytocin and infundin were all found to inhibit high or low spontaneous motility of the stomach, small intestine and colon. They likewise inhibit the hypermotile gut of insulinized animals. The inhibition begins in 15 to 20 seconds (before the blood sugar level is modified) acting probably through a direct paralysis of the motor mechanism of the gut. Modifications in carbohydrate metabolism are involved apparently in the later phase of the inhibition.

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CUTANEOUS RESPIRATION IN MAN

II. The Effect of Temperature and of Relative Humidity upon the Rate of Carbon Dioxide Elimination and Oxygen Absorption

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Received for publication May 31, 1930

Shaw, Messer and Weiss (1929) have shown that the principal factor influencing the rate of carbon dioxide elimination and oxygen absorption by the skin is the temperature of the air in contact with the skin. In their experiments a rise in temperature was accompanied by an increase in the sweat excreted by the skin, and therefore by an increasing humidity of the air surrounding the skin. The present investigation was undertaken to evaluate the effect of temperature and of humidity as independent factors affecting gas exchange through the skin.

Method. The subject lay on a couch in a comfortable position, with the entire arm in a plethysmograph. The experiment lasted for three or four hours. Gas samples were withdrawn from the plethysmograph, and the change in the percentage composition of the carbon dioxide and oxygen multiplied by the total volume of gas in contact with the skin gave the volume of carbon dioxide and oxygen which had been gained or lost during a given time.

The apparatus and technical procedure were essentially the same as that employed by Shaw, Messer and Weiss (1929). It was found advantageous to substitute a glass plethysmograph for the brass one so that the arm could be kept under observation throughout the experiment.

In studying the effect of temperature every precaution was taken to exclude all other influences upon the rate of carbon dioxide and oxygen diffusion through the skin. The same subject was used in all the experiments, thereby eliminating individual variations; the conditions of the room and the clothing were regulated to maintain a constant temperature of the air next to the skin of the body as a whole, thereby preventing circulatory changes of a general nature which might affect the circulation of the arms; the air next to the skin of the arm was kept at the saturation point to eliminate the effect of variations in humidity by means of a moist woolen stocking worn on the arm. Since the air in the system became saturated very rapidly, the cooling effect of evaporation from the stocking was of short duration. In the humidity experiments the temperature of the air in the

plethysmograph was held constant, while the relative humidity was raised from 20 per cent to 80 per cent.

In order to control the humidity it was necessary to substitute for the mercury pump described by Shaw, Messer and Weiss (1929) a blower immersed in heavy oil, which propelled the air through a condensation unit. This is illustrated in figure 1. The relative humidity of the air in the system could be regulated by altering the circulation rate—the more rapidly the air passed through the condensation coil the lower the humidity and *vice versa*. In order to maintain the air in the plethysmograph at the

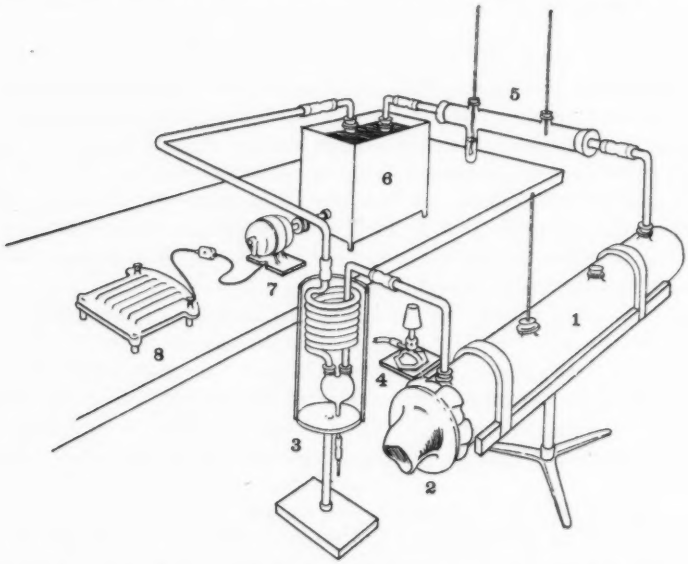

Fig. 1. Apparatus for control of relative humidity connected to plethysmograph. *1*, plethysmograph; *2*, rubber cuff; *3*, condensation coil; *4*, gas burner; *5*, wet and dry bulb thermometers; *6*, blower immersed in bath of oil; *7*, motor; *8*, rheostat.

desired temperature it was necessary to warm the air returning from the condensation coil by a gas flame applied to the inlet tube. The relative humidity of the air in the plethysmograph was estimated from readings of wet and dry bulb thermometers inserted in the outlet tube.

The maximum capacity of the blower was 50 liters per minute, corresponding to a relative humidity of about 20 per cent. By reducing the rate of delivery, relative humidities up to 80 per cent were obtained.

EXPERIMENTAL RESULTS. *A. Temperature effects.* The experiments on the temperature control of carbon dioxide elimination and oxygen ab-

sorption through the skin were done, as stated above, in an atmosphere completely saturated with moisture. Curve *A* of figure 2 shows the volume of carbon dioxide which passes through the skin of the arm of J. S. P.,

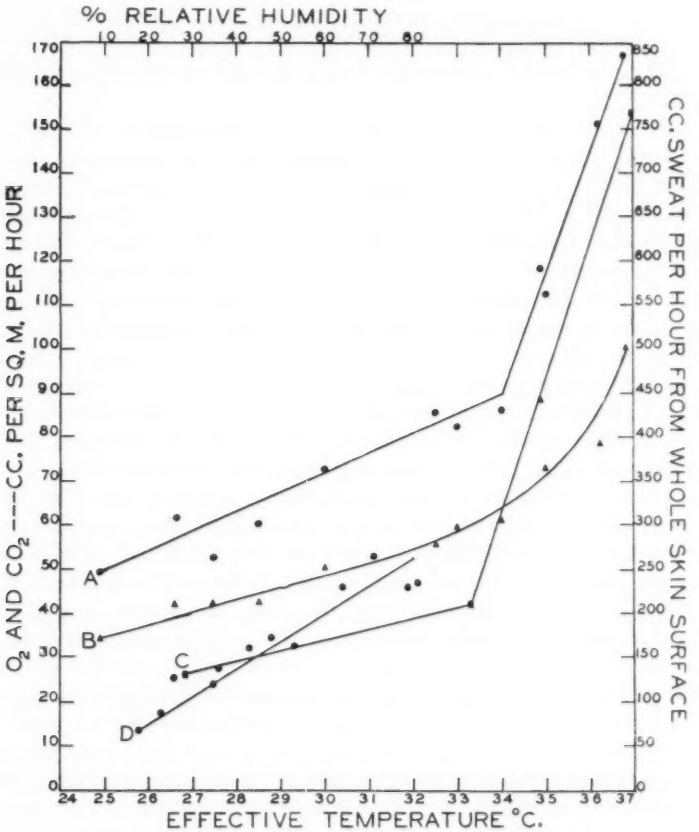

Fig. 2. Curve *A*, effect of temperature on rate of carbon dioxide excretion. Curve *B*, effect of temperature on rate of oxygen absorption. Curve *C*, effect of temperature on rate of sweat excretion. Curve *D*, effect of relative humidity on rate of carbon dioxide excretion.

calculated in cubic centimeters per hour per square meter of skin surface, correlated with an increasing temperature of the air surrounding the skin. The ratio of carbon dioxide eliminated to oxygen absorbed varied from 1.27 to 1.92, with an average value of 1.48. At 34°C. there is a break in the

curve, due to the onset of sweating. This factor will be discussed later. Curve *B* shows the volume of oxygen absorbed.

B. Humidity effects. The effect of relative humidity in controlling the rate of carbon dioxde excretion is shown in figure 2, curve *D*. These experiments were done while the arm was surrounded by air having a constant dry bulb temperature of 33°C. The ratio of carbon dioxide eliminated to oxygen absorbed varied from 1.03 to 2.31, with an average value of 1.79.

Discussion. It will be observed that the temperature curve for carbon dioxide elimination (fig. 2) is a straight line with one break, which occurs at 34°C.; whereas the temperature curves previously reported by Shaw, Messer and Weiss (1929) are concave upward. This difference is undoubtedly due to the fact that, in the experiments of Shaw, Messer and Weiss, since there was no humidity control, the relative humidity steadily increased with the rising temperature, due to the water given off by the skin. As a consequence, the effect of increasing relative humidity was added to the effect of temperature. Curves *A* and *D* in figure 2 show the effect of these factors acting separately.

The factors which control both the elimination of carbon dioxide and of sweat through the skin are strikingly similar. Houghten and Yagloglou (1923) found that at any constant temperature exceeding 90°F. the rate of sweat production varies with the relative humidity; and that with a constant relative humidity the rate of sweat production varies with the temperature. From this we may conclude that the rate of sweat production depends both on the temperature and on the relative humidity of the air. Houghten and Yagloglou have, therefore, devised the so-called "effective temperature" scale which is an index of the combined effect of both temperature and humidity upon the human body. According to their definition the "effective temperature is an index of the sensations of warmth or cold felt in response to temperature, humidity, and movement of the air. When the dry and wet bulb temperatures and the rate of air movement are known, the effective temperature can be computed from charts or tables." For example, human beings will tolerate a dry bulb temperature of 105°F. with a relative humidity of 43 per cent, or a dry bulb temperature of 97°F. with a relative humidity of 68 per cent, with the same degree of comfort as air which is saturated at 90°F. In other words, the dry bulb temperature alone is no criterion of our feeling of comfort. The relative humidity must always be taken into account.

It is of interest to compare the rate of carbon dioxide excretion with the rate of sweat excretion by the skin when correlated with the effective temperature, as shown in figure 2, curves *A* and *C*. Curve *C* was taken from Houghten and Yagloglou (1923) and shows the rate at which sweat is excreted by the skin of the entire body under the influence of effective tem-

perature changes. It will be observed that at 33.3°C. there is a very marked increase in the rate of sweat excretion. This increase has been attributed to the onset of visible perspiration or sweating. Below 33.3°C. the perspiration is invisible.

A similar increase in the rate of carbon dioxide excretion takes place at 34°C. The critical temperature at which the rate of carbon dioxide excretion shows this marked increase is thus identical, within the limits of the experimental error, with the critical temperature of sweat excretion.

Schierbeck (1893) and von Willebrand (1902) established a critical temperature at 33°C., above which the rate of carbon dioxide excretion increased very rapidly. Below 33°C., however, they found the rate of carbon dioxide elimination to be constant and independent of temperature. Their failure to detect an increasing rate of carbon dioxide excretion with rising temperatures below 33°C. was undoubtedly due to the inaccuracy of the analytical methods employed. Aubert (1872), however, did detect an increased rate of carbon dioxide elimination at temperatures below 33°C.

The curve for oxygen absorption (*B*, fig. 2) fails to show the critical break at 33.4°C. which characterizes the curve for carbon dioxide elimination. This is undoubtedly due to the fact that the increased vascularization of the skin caused by increasing the temperature, results in a highly arterialized blood supply with a high oxygen tension. In this event the increased area of contact between the blood and the air caused by high temperatures is, to a large degree, neutralized by the diminished pressure head under which the oxygen passes from the air into the blood. On the other hand the variations in carbon dioxide tension of blood, which may be more or less arterialized according to the rate of flow, will be comparatively slight, owing to the nature of the carbon dioxide dissociation curve. The carbon dioxide tension difference between the blood and air will, therefore, not be greatly affected by alterations in blood flow, as is the case with the oxygen tension.

The increased rate of cutaneous respiration which accompanies a rise in the effective temperature, must be due almost entirely to an increasing utilization of the capillaries of the skin, thereby increasing the area of contact between the air and the blood as the latter flows through the superficial strata of the skin.

Houghten and Yagloglou (1923) have shown that, after the critical temperature (33.4°C.) has been passed, the excretion of sweat is proportional to the increase in effective temperature up to 44.5°C., which is the highest temperature their experimental subjects could tolerate. It is evident, from inspection of curve *A*, figure 2, that, after the critical temperature (34°C.) has been passed, the excretion of carbon dioxide is also proportional to the increase in effective temperature. Though we have not sub-

jected the skin to a higher effective temperature than 36.8°C., we may easily calculate the rate of carbon dioxide excretion at higher temperatures, on the assumption that the proportionality persists to the limit of endurance, as is the case with water excretion. At an effective temperature of 44.5°C., therefore, we should expect to find that the rate of carbon dioxide excretion was 379 cc. per square meter of skin surface per hour. Under normal conditions of bodily comfort the effective temperature of the air next to the skin is about 28°C., at which temperature our subject would excrete about 63 cc. of carbon dioxide per square meter per hour. At an effective temperature of 36.8°C., the highest temperature attained in our experiments, the rate of carbon dioxide excretion was 265 per cent of the normal rate, and at an effective temperature of 44.5° we may assume that the rate of excretion would be 600 per cent of the normal.

The effect of the relative humidity upon the rate of cutaneous respiration was determined, as stated above, by holding the arm in air having a constant dry bulb temperature of 33°C., while the relative humidity was changed. At a relative humidity of 20 per cent our subject excreted about 15 cc. of carbon dioxide per square meter of skin surface per hour, which rose to 52 cc. at a relative humidity of 80 per cent. In these experiments the skin was exposed to the air without any covering, as was found expedient in the experiments in air saturated with water, and so far as we could observe the skin remained perfectly dry. The increase in carbon dioxide output with increasing humidity was undoubtedly due to a diminished rate of evaporation, which in turn produced an effect which was equivalent to raising the temperature.

SUMMARY

1. The rate of carbon dioxide excretion and oxygen absorption through the skin of the arm was observed *a*, at different temperatures and a constant relative humidity, and *b*, at different relative humidities and a constant temperature.

2. The rate of carbon dioxide excretion increases with rising temperature. At 34°C., there is a critical point above which the rate of carbon dioxide excretion becomes six times as rapid per degree of temperature increase as at temperatures below 34°C.

3. The oxygen absorption bears an approximately constant ratio to the carbon dioxide excretion until 34°C. is reached, at which point the ratio of carbon dioxide excretion to oxygen absorption becomes greatly increased.

4. The rate of cutaneous respiration increases with the relative humidity due to the thermal effect of evaporation.

5. The relationship between the rate of carbon dioxide excretion and the rate of sweat excretion through the skin has been shown.

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THE VASOMOTOR CONTROL OF THE LIVER CIRCULATION

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Received for publication June 6, 1930

The size and vascularity of the liver must make it an important factor in regulating the volume and pressure of the blood in general circulation; its own various metabolic processes, as first suggested by Claude Bernard in connection with carbohydrate mobilization, may be affected by the amount of blood passing through it; yet in spite of these important bearings our knowledge of the precise details of the vasomotor control of the hepatic circulation is in certain important respects quite unsatisfactory. This is the justification for an addition to the small literature which already exists in regard to this subject.

METHOD. Cats have been used for all of the experiments to be described here; these were under chloralose anesthesia at the time of determining the liver reactions. The chloralose was occasionally given by stomach-tube or intraperitoneal injection; but more often the animal was first put under with ether and as soon as unconscious the chloralose was injected into the small intestine. The ether could be discontinued almost immediately and must have been quite thoroughly eliminated from the body during the half-hour or so required to insert the carotid and tracheal cannulae and complete the other preparations for the experiment. Nevertheless some effect of the ether which was quite beneficial for these experiments seemed to remain and it was for this reason that this form of anesthesia was chiefly used; pure ether anesthesia seemed to have too deadening an effect on the liver reflexes; under chloralose alone on the other hand (in the concentration recommended, 0.1 gm. per kilo) the animal was too jumpy and irritable, in that the slightest afferent stimulation caused such widespread reflex activity and especially such respiratory disturbance that the plethysmographic record would be gravely distorted. For a time it was attempted to eliminate these by the use of curare and artificial respiration; but the results did not seem as satisfactory as those obtained from the simple combination of the preliminary ether and subsequent chloralose anesthesia alone; this seemed to produce a preparation with sensitive visceral reflexes undisturbed by too vigorous general muscular activity. A few efforts were made to work with decerebrate animals; but these suffered from the same drawbacks as those under pure chloralose anesthesia, only in an

exaggerated form; so that in order to secure undistorted plethysmographic records of the liver volume it was necessary to dull the edge of the general skeletal reflexes with a few whiffs of ether before each stimulation.

During the second or third hour after giving chloralose the animal will settle down, without any preliminary ether, into a less jumpy condition in which afferent stimulation does not produce such widespread muscular activity. But, and we think it is important to emphasize this, the liver reflexes will usually have disappeared at the same time. We have been able to obtain consistent successes in regard to some of the responses, notably reflex dilatation in the liver, only by reducing the interval between the beginning of anesthesia and the securing of the response as much as possible. In our hands the use of ether and chloralose effects the proper combination of quickness and desired depth of anesthesia better than anything else we have tried.

The condition of the circulation in the liver has been deduced from records of its volume. The plethysmograph which we have used is one which includes the entire organ; see figure 1. For the fashioning of it a wooden model was carved of the liver frozen *in situ*; upon this was then modeled a shell of thin copper; this has the shape, essentially, of a flattened bell; and after the exposure of the liver by a ventral mid-line incision it is lowered over the entire organ. The edges of the bell are shaped so as to conform to the dorsal body wall and are notched on the anterior and posterior faces so as to prevent occlusion of the hepatic artery and the portal and hepatic veins. The free edges are bound with surgical tape and the whole instrument is coated inside and out with paraffine to prevent the viscera from having any contact with metal. A small brass tube is fixed in the ventral (top) part of the bell for the attachment of tubing to connect with a tambour recorder.

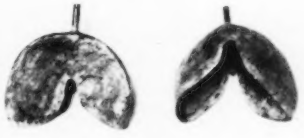

Fig. 1. Liver plethysmograph

When the instrument is in place its free edges usually conform so exactly to the dorsal body wall as to make an air tight junction; if, however, the cat is of such a size that such a fit does not occur automatically, a seal is provided by filling the abdominal cavity with enough salt solution or mineral oil to cover the edges.

As we have used this plethysmograph the portion of the esophagus between the diaphragm and the stomach has usually been included along with the structures of the lesser omentum under the notches so that the fundus and the lesser curvature of the stomach have been drawn tightly against the posterior surface of the instrument. This has been done rather than subject the liver to the trauma involved in ligating and cutting

the esophagus; because controls have shown this portion of the esophagus to have no effect on the records, viz., all of the more important reactions have been duplicated after eliminating this portion of the esophagus; but more crucially, every reaction which has been attributed to a vaso-motor mechanism within the liver has been abolished by the technically very simple and undisturbing operation of merely cutting the nerves in the hepatic plexus and leaving all other conditions of the set-up undisturbed.

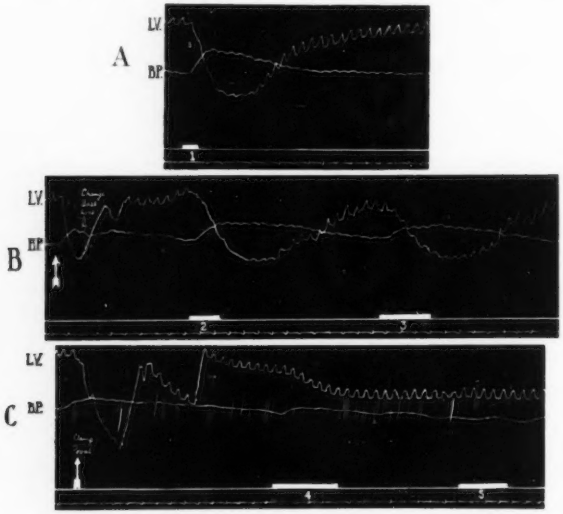

Fig. 2. Stimulation of the hepatic nerves along the hepatoduodenal artery: A, normal. B, with the hepatic artery clamped, at the arrow, i.e., showing constriction of the terminations of the portal vein. C, with the portal vein clamped, at the arrow, i.e., showing constriction of the arterioles.

The first parts of B and C show adjustments of the volume recorder to compensate for the change in liver volume caused by shutting off its blood supply by way of the artery or vein.

In all records, *B.P.* and *L.V.*, are blood pressure and liver volume respectively; time record, 5 second, intervals.

Results. 1. *Stimulation of the hepatic (postganglionic) nerves.* The nerves which accompany and envelope the hepato-duodenal artery were separated from it by gentle dissection so that shielded electrodes could be applied to them without interfering with the flow of blood in the vessel; they were then crushed, or ligated and cut, central to the electrodes and stimulated with a mild tetanizing current.

Such stimulation causes a marked decrease in liver volume, associated with a rise in the arterial (carotid) blood pressure; see figure 2A. The

effect on the general blood pressure has been analyzed in a previous report and need not be gone into here (Griffith, York and Zachmys, 1928).

This decrease in the volume of the liver must indicate constriction of its blood vessels; whether this applies to the terminations of both the hepatic artery and the portal vein has been one of the most disputed points of this obscure subject; our evidence indicates that both are affected.

Constriction of the portal venules: figure 2B shows the decrease in liver volume resulting from stimulation of the hepatic nerves while the hepatic artery is clamped. The clamp was applied at the arrow and the first part of the record is taken up with adjustment of the volume recorder necessitated by the shrinkage in the size of the liver which results when its arterial supply is obstructed. The remainder of the record shows the additional, temporary constriction which can be produced in such a liver during stimulation of its nerves.

It might be argued that although the artery is clamped its terminations need not have completely emptied themselves and that this occurring when the nerves are stimulated would account for the additional decrease in the volume of the liver. As evidence against this the record (2B) shows that upon cessation of the stimulation the liver returns promptly to the pre-stimulation volume and can be again constricted, to as great an extent as initially, by a second stimulation; indeed, we have repeated this in some instances four times, and presumably could do so still oftener, with no diminution in the magnitude of the response. This would seem to establish the control of the hepatic nerves on the calibre of the portal venules.

Constriction of the hepatic arterioles: previous evidence is entirely in agreement as to the constrictor action of the hepatic nerves on the hepatic arterioles; and our own work substantiates this as may be seen from the record reproduced in figure 2C. In this case the nerves were stimulated while the portal vein was clamped; the clamp was applied at the arrow and, again, the first part of the record is taken up with the adjustments of the volume recorder made necessary by the great decrease in the volume of the liver when its portal supply is interfered with. Even after this first, pronounced shrinkage of the liver is over it will be seen that there is a continual, gradual diminution in its volume; this has continued, in our experience, as long as the clamp has been left on the portal vein and we attribute it to the concomitant, progressive fall in the arterial blood pressure (the liver being supplied only through its artery) which is due to the accumulation of blood in the obstructed portal system. The effect of the stimulation of the hepatic nerves is therefore superimposed on this gradual decline; but it will be seen that the first stimulation produces an unmistakable and prompt, additional constriction of the liver. The record is typical, however, in showing that the liver fails to return to its pre-stimulation volume; this might be due to the fact, just mentioned, that the arterial

blood pressure, which is the only force keeping the liver distended during this time, is gradually falling; but it is less easy to account for the fact that a second stimulation is apparently without effect. This might imply, either that the constriction of the arterioles, resulting from the first stimulation, is long-lasting; or that the arterial pressure is, by this time, too low to distend the relaxed capillaries.

We conclude, therefore, that the post-ganglionic fibers of the hepatic plexus exercise control over the terminations of both the hepatic artery and portal vein; and the latter is so clear-cut, in our experience, that we are unable to understand the difficulty which previous workers have had in obtaining it.

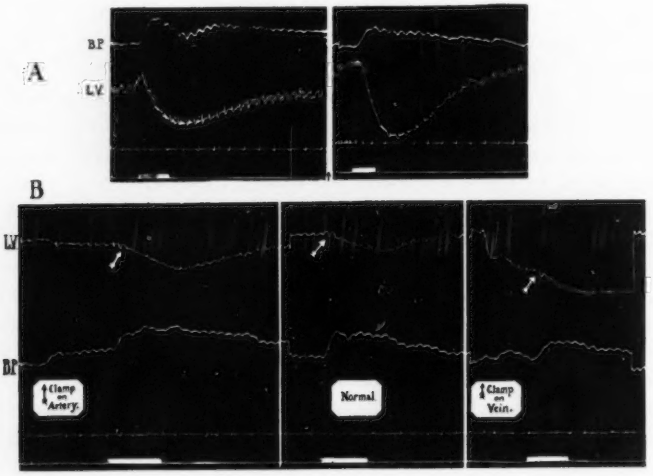

Fig. 3. Stimulation of the left splanchnic nerve: A, left adrenal removed between the two records. B, first stimulation with the hepatic artery clamped. Second stimulation, normal. Third stimulation, with the portal vein clamped. *B.P.* and *L.V.*, blood pressure and liver volume, respectively; time, 5 second intervals.

2. *Stimulation of the splanchnic (pre-ganglionic) nerves.* There would be no occasion to consider the splanchnics separately and apart from their post-ganglionic continuations in the hepatic plexus except for the fact that Burton-Opitz (1912) has claimed without, so far as we can find, subsequent amendment, that splanchnic stimulation affects the circulation in the liver only by virtue of such changes as it produces in the general blood pressure. And in spite of the unusual dissociation which this implies between the pre- and post-ganglionic fibers it has been incorporated into so authoritative a monograph as that of Wiggers.

Our own experience is totally opposed to such a view, as may be seen from figure 3A. In the first record of this figure there appears a very slight and transient increase in the volume of the liver, accompanying the rising blood pressure, but this occurs only rarely and when it does, as in this instance, it promptly gives way to a pronounced constriction as the stimulation proceeds. The second record of this figure was taken from the same animal after removing the adrenal on the stimulated side and shows that the constriction of the liver is a true vaso-motor effect and not due to liberation of adrenin as a result of the stimulation.

Figure 3B shows that the splanchnic, like its post-ganglionic continuations in the hepatic plexus (as described in the preceding section) exercises control over the terminations of both the hepatic artery and portal vein; the first record shows the effect obtained by way of the portal vein while the artery is clamped; the middle reaction is normal; and the third shows constriction of the arterioles while a clamp is on the portal vein. These are so similar to those described in the preceding section that they need no further comment.

Fig. 4. Stimulation of the left splanchnic before and after cutting the post-ganglionic nerves to the liver. Lower record blood pressure, upper record liver volume; time, 5 second intervals.

It might be felt that this decrease in the volume of the liver which results from splanchnic stimulation, and especially that by way of the portal vein, could be attributable to the general visceral constriction preventing the normal flow of blood into the liver and hence a collapse in its volume. Figure 4 shows that this can account for very little of the effect; for merely cutting the post-ganglionic fibers in the hepatic plexus, but interfering in no wise with any other portion of the splanchnic vascular bed, prevents all but a negligible decrease in liver volume—a residual decrease which may be due to the diminished portal supply which has just been mentioned.

It must be concluded, therefore, that the pre-ganglionic fibers in the splanchnic trunk produce results identical with those of the post-ganglionic fibers in the hepatic plexus.

3. *Peripheral vagus stimulation.* In order to avoid cardiac inhibition, with its accompanying fall in blood pressure, during the vagus stimulation we have *A*, stimulated the left vagus in the neck after completely denervating the heart according to Cannon's method; and *B*, stimulated both vagi simultaneously *a*, below the heart in the thorax, and *b*, below the diaphragm.

In all cases the effective functioning of the peripheral parts of the vagus

trunks was evident from the vigorous contractions of the gut which occurred during the stimulations. But in no instance was the volume of the liver affected in any way by any strength of stimulus. It therefore seems necessary to conclude that the vagi do not carry vaso-motor fibers to the liver.

4. *Reflex control of the liver circulation.* A. Vaso-constrictor reflexes. Francois-Franck and Hallion (1897a) had observed constriction within the liver as a result of appropriate stimulation of the central vagus and crural nerves. Since then the phenomenon has apparently received no attention. The subsequent discovery of the part played by adrenin in vaso-motor activities and the known sensitivity of the liver to it raised the question as to how much if any of the constriction that was observed

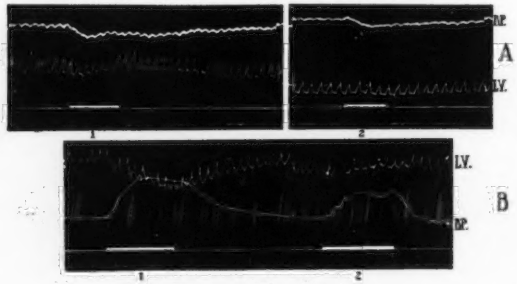

Fig. 5. Reflex (sciatic) vasodilatation (upper record, A) and vasoconstriction (lower record, B) in the liver, abolished by cutting the post-ganglionic hepatic nerves. In both instances the nerves were cut immediately after the first stimulation and 10 minutes before the second. Vasodilatation, stimulus, Harvard coil, 12 cm. Vasoconstriction, stimulus, Harvard soil, 4 cm.; A, both adrenals out. *B.P.* and *L.V.*, blood pressure and liver volume, respectively; time, 5 second intervals.

as a consequence of afferent stimulation might be due to a reflexly augmented secretion of this hormone.

Our chief contribution in this matter is to have tested this possibility; figure 5B shows that the constriction in the liver which follows upon stimulation of the central end of the sciatic is completely abolished by merely cutting the post-ganglionic fibers in the strands that accompany the hepatoduodenal artery; the record shows that after this denervation the volume of the liver passively follows the general blood pressure in spite of the fact that the adrenals are still intact and capable of normal functioning. It will be shown later that the adrenals do play a part in the reaction of the liver to asphyxia; but the stimulation employed to produce these responses was apparently too weak or of too short duration to bring them into activity. At any rate reflex constriction within the liver is proven to be a true vaso-motor effect.

B. Vaso-dilator reflexes. Francois-Franck and Hallion (1897a) observed dilatation in the liver as a result of appropriate stimulation of the central end of the vagus; we have confirmed this and have also obtained, and chiefly studied it, as a result of depressor stimulation of the central end of the sciatic.

This dilatation of the liver accompanying reflex depressor reactions is not mechanical in origin; i.e., it is not due to dilatation of the other visceral arterioles allowing a greater volume of blood into the portal system to engorge the liver. At least it is possible to demonstrate active dilatation through the hepatic artery alone, as in figure 6A which was obtained with the portal vein entirely shut off so that variations in blood supply through the portal system could have no effect on the volume of the liver.

It has been impossible with our technique to devise an equally crucial demonstration of active dilatation on the terminals of the portal vein. It

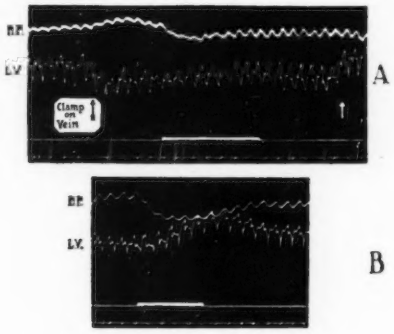

Fig. 6. Reflex (sciatic) dilatation in the liver: A, on the terminations of the artery after clamping, at the arrow, the portal vein. B, after cutting both vagi in the neck.

may be said, however, that the dilatation which takes place after occlusion of the portal vein (i.e., on the terminals of the hepatic artery alone) is much less in magnitude (fig. 6A) than that occurring in the normal liver (fig. 6B). And, finally, no dilatation takes place in the liver during depressor reactions after cutting the post-ganglionic fibers in the hepatic plexus; the liver volume then follows passively the fall in general blood pressure, as may be seen in figure 5A. This is conclusive proof that the reaction is a true vaso-motor reflex within the liver and not a passive modification of its volume as a result of changes in blood flow in some other part of the portal bed.

The vagi play no part in this dilator reaction; this would follow almost as a matter of course from the evidence already given in a preceding section that they have no effect on the volume of the liver when stimulated di-

rectly. Figure 6B shows that this reflex dilatation may occur in the liver after both vagi have been cut in the neck; the evidence is therefore conclusive that these nerves do not carry vasomotor fibers to this organ.

Both constriction and dilatation in the liver must therefore be mediated through the splanchnics. Direct stimulation of these nerves or of their post-ganglionic continuations into the liver has always produced constriction. This must mean that dilator fibers if present are difficult to excite

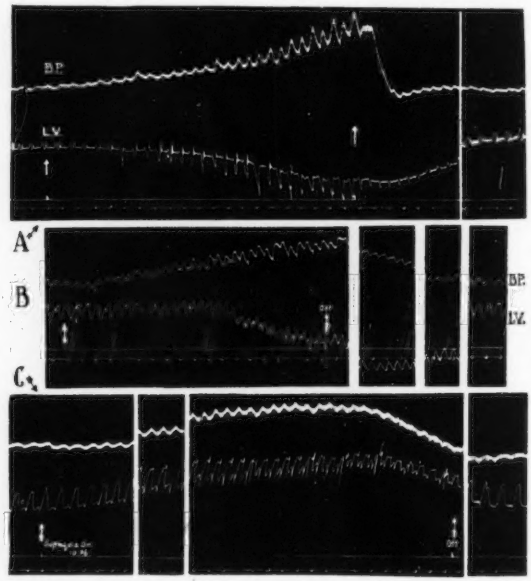

Fig. 7. The effect of asphyxia on the volume of the liver: A, normal. B, after cutting the post-ganglionic hepatic nerves. C, after cutting the nerves and removing both adrenals.

Asphyxia begun and ended at the arrows; *B.P.* and *L.V.*, blood pressure and liver volume, respectively; time, 5 second intervals.

by direct stimulation; or that their effect is over-shadowed by the concomitant constriction; or that dilatation within the liver is due entirely to reflex inhibition of the prevailing constrictor tone—ambiguities which unite the liver with the other viscera.

It may be mentioned that this dilatation proved for a time to be very elusive; it is never possible to be certain of obtaining it; but it may be secured with fair regularity by being quite careful as to the treatment of the animal. As was mentioned in a previous section the anesthesia must not be too deep; and the response is more certain at the very beginning of an

experiment and even then the first few stimulations may be the only successful ones. Although the reaction may fail under what seem to be the best conditions, it seems to be correlated with the height of the blood pressure; at least it is hopeless to expect to obtain it after the blood pressure has fallen in the course of a long experiment. All of this favors the idea that the reaction is essentially an inhibition of constrictor tone and is therefore only to be obtained when this is high.

5. *Asphyxia.* In these experiments asphyxia has been produced by rebreathing the air in a small balloon attached to the trăcheal cannula.

The typical reaction is shown in figure 7A; as the blood pressure rises there is pronounced constriction in the liver; and this persists quite beyond the point where the heart begins to fail and even until just before death if the reaction is pushed so far.

After cutting the post-ganglionic fibers in the hepatic plexus the course of the asphyxial reaction is as shown in figure 7B. The difference between this and the normal is that the constriction in the liver is considerably delayed; so that instead of starting immediately and with the beginning of the rise in blood pressure, the liver does not begin to show signs of constriction until about the middle of the blood pressure reaction; indeed it usually dilates under the force of the mounting blood pressure during this time. At about this point, however, the liver invariably begins to constrict quite vigorously and abruptly and usually to as great an extent as normally.

This delayed constriction which must in the normal liver reinforce and sustain the initial reflex is due to the adrenals. This is evident from figure 7C which shows that after cutting the hepatic nerves and also removing both adrenals the volume of the liver passively follows the general blood pressure.

6. *Hemorrhage.* From the beginning of this work we considered it reasonable to expect that the relatively large resources of the hepatic reservoir would be called upon to help sustain the general blood pressure in the early stages of hemorrhage. For two years or more we were repeatedly disappointed in realizing this expectation. During this time we made a habit of terminating many of the experiments with hemorrhage; and invariably the liver showed no sign of doing other than passively following the falling blood pressure. Only recently did it occur to us to try the effect in a fresh preparation and with results that were as beautiful as they were gratifying.

The records of figure 8 were taken as blood was slowly withdrawn from the femoral artery into a heparinized syringe and then slowly reinjected. Figure 8A shows the reaction of the normal liver after a slight preliminary fall, the blood pressure is maintained almost constant while the liver constricts vigorously; reinjection restores both the blood pressure and liver

volume to normal. On the other hand, if the post-ganglionic fibers in the hepatic plexus are cut it is never possible to get the liver to behave other than as shown in figure 8B; here it follows passively the declining blood pressure.

Figures 8A and B were taken in rapid succession; it is interesting to note that whereas in the first record only 10 cc. of blood were taken from the animal, the decrease in liver volume is greater than in the second, where the amount of blood lost was 15 cc. This is additional proof that the decrease in volume in the first case is active and not dependent on mere loss of blood from the body.

It may be mentioned that the spleen was tied off during these reactions so that it could play no part in them.

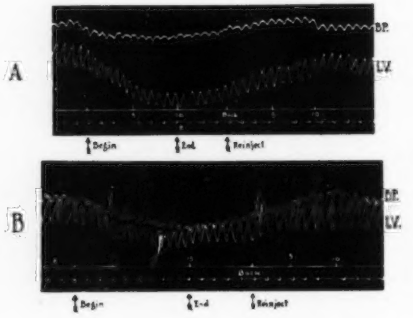

Fig. 8. The effect of hemorrhage on the volume of the liver: A, normal (with the spleen tied off). B, after cutting the hepatic nerves. The hemorrhage was begun and ended at the first and second arrows; reinjection begun at the third arrow; 10 cc. withdrawn in A, 15 cc. in B; *B.P.* and *L.V.*, blood pressure and liver volume, respectively; time, 5 second intervals.

Discussion. Most of the previous work and controversy in regard to the hepatic circulation has centered around the question as to the relative amount of control exercised by the hepatic nerves over the portal venules and hepatic arterioles respectively. Francois-Franck and Hallion (1895b) in one of the first, and in many ways even yet the most complete work on the circulation in the liver, secured plethysmographic records and pressure changes in the hepatic artery and portal vein which indicated that stimulation of the splanchnics or of their post-ganglionic ramifications along the hepatic artery constricted both the arterioles and venules. A little later the action on the venules was confirmed by Schmid who measured the pressure and blood flow (stromuhr) in the portal vein.

Thus the matter stood until Burton-Opitz reported failure to obtain any change in the flow (stromuhr) or pressure in the portal vein as a conse-

quence of stimulating the fibers of the hepatic plexus (1911b); a later observation of his, however, (1912d) that adrenalin produced constriction of the portal venules, led him to re-examine the question (1914) and to conclude that although their action on the terminations of the portal vein was weaker than on the arterioles, nevertheless the post-ganglionic fibers of the hepatic plexus do control the flow of blood in both systems. And as further evidence that the two systems are separate and distinct, he deduced from his records of the pressure changes within them that while both rises in pressure closely agree with the strength and duration of the stimulation, yet the arterial change conformed to it the more closely, in that the pressure within the artery regained its normal level before the portal had become adjusted.

All later work has only served to confirm this impression, introduced by Burton-Opitz, that the nervous control of the portal terminals is less alert and positive than that of the arterioles. Macleod and Pearce were the next to add evidence in its support. They attempted to measure the rate of flow through the liver by collecting all the blood escaping from it at any given time. The inaccessibility of the organ naturally made such a method difficult of application. To do it they passed a cannula up the vena cava to the level of the hepatic veins; when it was desired to get the rate of flow through the liver the vena cava anterior to the end of the cannula was clamped so that all blood coming through the organ was diverted back through the cannula to be collected in a volume recorder. We have mentioned in a previous section the necessity of having the animal in the best possible condition in order to assure success in obtaining many of the vaso-motor reactions within the liver; although we have never failed to secure this particular reaction under any conditions involved with our technique, it may be well to emphasize the drastically unphysiological character of the set-up employed by these investigators. With it, however, they were able to demonstrate a constriction of the terminals of the hepatic artery when the strands of the hepatic plexus were stimulated; such stimulation had no effect on the blood flow through the liver, however, after the artery was clamped. But as they were able to demonstrate constriction on the terminals of the portal vein with the use of adrenalin, they concluded that although a vaso-motor mechanism must exist there it must be quite weak and ineffective. Their paper is also interesting for an ingenious theory as to the effect of certain mechanical arrangements within the Glisson capsule on the flow of blood in the arterioles and venules, but which need not be gone into here.

Edmunds is the last, so far as we know, to have been concerned with this question. He measured simultaneously the volume of the liver and the portal, caval and general arterial blood pressures; and found that during the rise of general blood pressure resulting from splanchnic stimulation

there was a rise in the portal pressure; since the pressure in the cava did not change, it was concluded that the rise of portal pressure must indicate constriction of the portal venules. Disconcertingly, however, the liver volume *increased* simultaneously with the above reactions, a matter quite difficult to reconcile with the idea of arterial and venous constriction within an organ. And although Edmunds attempted to account in a satisfactory manner for this apparently anomalous reaction, one is again left with the impression that constriction of the portal venules as a result of nerve stimulation is relatively ineffective in controlling the mass of blood within the liver.

In conclusion, our own evidence on this point is a complete confirmation of the original position of Francois-Franck and Hallion and indicates that vaso-motor mechanisms of force and potency exist in connection with the terminations of both the arterial and venous inflows into the liver; and there has been nothing in our experience to suggest any explanation of the difficulty which others have had in obtaining prompt and vigorous constriction of the terminations of the portal vein.

When we pass to consider the central connections of the vaso-constrictor fibers of the hepatic plexus it is surprising to find that Burton-Opitz' dictum, that the splanchnics are without effect except indirectly through their effect on the general blood pressure has passed without challenge. Our own results have been seen to be quite opposed to such an idea; indeed, Francois-Franck and Hallion in their beautiful and too much neglected work, have traced the vaso-constrictor outflow for the liver back to the 6th dorsal to 2nd lumbar spinal roots (1911a) and showed, as have we, that the whole mechanism can be thrown into action reflexly, as indeed there could be no *a priori* reason for doubting.

Our opinion as to the absence of vaso-motor fibers in the vagus might be considered to have confirmation in previous work in the sense that the earlier reports have been mutually antagonistic. Francois-Franck and Hallion in one of the few statements with which we are forced to disagree, report (1896) dilatation, whereas Neubauer (1914) obtained constriction in the liver as a result of stimulating these nerves peripherally.

The reflex employment of this vasomotor mechanism has been very little studied. We know of no previous work on the reaction of the liver during hemorrhage; and Francois-Franck and Hallion are the only ones to have been interested in its response during pressor and depressor stimulation; to this work of theirs we have been able to add little except in the way of proof that the changes which take place in the volume of the liver are not due to engorgement or depletion of the organ as a result of vasomotor activity elsewhere. These workers were also the first to study the liver during asphyxia (1897) and our observations are a confirmation of theirs for the first part of the reaction; but whereas we find the liver to remain

constricted until nearly the point of death, they reported it to begin to dilate and become engorged with blood as soon as the heart began to fail. This difference, however, is probably due to the fact that they worked with curarized animals and brought on the asphyxia by suspension of the artificial respiration; which would disturb the normal pressures within the thorax upon which the proper movement of blood is so dependent; indeed we have found it possible to cause great engorgement of the liver by slightly too vigorous artificial respiration alone.

Much the same objection might be made to the dilatation observed by Neubauer (1912) during asphyxia brought on by clamping the trachea; here again an engorgement of the liver could easily result from the improper movement of blood due to the changed pressure relations within the thorax.

It is impossible, however, to apply such criticism to the dilatation during asphyxia which has been reported quite recently by Mattson; the technique in this case seems flawless and is superior to our own in the avoidance of an anesthetic. We can only say that what he reports as typical is a reaction which we have observed only occasionally and have felt forced to regard as a failure of the normal mechanism to function properly; due, perhaps, to an atonic condition of the vasomotor system which we have been inclined to attribute in our experiments to improper anesthesia, but which might result from various causes. At least, a dilatation of the liver *pari passu* with the mounting blood pressure has all the appearance of a passive reaction; a decrease in volume under these conditions might result from mechanical disturbances but since it is modified and abolished by merely cutting the hepatic nerves and removing the adrenals it would hardly seem so; for these can have no effect on the mechanical pressures developed within the thorax and abdomen. But because this mechanism exists it does not follow that it will invariably respond although with us it has done so more often than not.

SUMMARY

By the use of a special plethysmograph which permits records to be taken of the volume of the entire liver the following have been established in regard to the vaso-motor control of the hepatic circulation:

1. Stimulation of the post-ganglionic fibers along the hepatoduodenal artery or of the pre-ganglionic fibers of the splanchnic nerve causes constriction of the terminations of the hepatic artery and of the portal vein.

2. Stimulation of the peripheral vagus has no effect on liver volume.

3. The liver constricts during pressor and dilates during depressor reflexes; both reactions are abolished by cutting the postganglionic fibers of the hepatic plexus.

4. The liver constricts powerfully during asphyxia; this is inaugurated

through its nerves and reinforced and sustained by the asphyxial secretion of adrenin.

5. Hemorrhage induces a contraction of liver volume which may be sufficient to sustain the blood pressure; this reaction, too, is abolished by section of the hepatic plexus.

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THE PREPARATION OF A SECRETIN CONCENTRATE

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Received for publication April 21, 1930

In the early part of 1927 our group undertook the problem of preparing a secretin concentrate not only because of its academic interest, but also with the hope that by obtaining a "purified" product, we might be able by its use to devise a human pancreatic function test. From time to time in connection with our articles on cholecystokinin, we (Ivy and Oldberg, 1927; Ivy and Oldberg, 1928; Ivy, Oldberg, Kloster and Lueth, 1928; Lueth and Kloster, 1928; Ivy, Kloster, Lueth and Drewyer, 1929) have reported that we have been able to obtain pancreatic responses with as small amounts as 0.2 mgm. of dry powder in thirty pound dogs. Such concentrates have been prepared in a number of different ways, but we have not published in detail any of these methods because they failed on frequent repetition to yield a uniform product. In the course of our work, Mellanby's (1928) method for the preparation of a secretin concentrate, using the "bile-salt precipitation principle," was given a trial by Mortimer and Ivy (1929) and was found in their hands as well as in the hands of Still (1930) not to be uniformly reliable, and was no better than some of our own methods which we considered of no definite value because of their lack of yielding a uniform product.

Still (1930) has recently published a report in which he used our principle (Ivy, Oldberg, Kloster and Lueth, 1928) of trichloracetic acid precipitation and added the method of adsorption to brucine. He obtained a highly active product (active in a 10 kgm. dog of 1 mgm. or less), but claimed only a 60 per cent uniformity for his procedure. We have tested his product and have confirmed his claims, which he has likewise done for our product.

In a paper on "The Preparation of a Cholecystokinin Concentrate," which precedes this paper, we have described the "1852" method which yields a secretin active in less than 1 mgm. doses. It is an expensive method and difficult to carry out because of the large volumes of acetone required.

In this paper we desire to describe the method that we have adopted

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for the preparation of a secretin concentrate active in less than 1 mgm. doses. It is reliable, rapid and relatively inexpensive.

METHOD. *The "S I and S II pH 1802" method:* In our description of the procedure we will start with the NaCl precipitate, the preparation of which we have previously described (Ivy, Kloster, Lueth and Drewyer, 1929) and which we call "A." This is the "new secretin" of Weaver, Luckhardt and Koch (1926), and is active on the pancreas in from 75 to 125 mgm. doses depending chiefly on the amount of salt present. We employ the hog's intestines because they are readily secured fresh and as much as fifty pounds of "A" precipitate, which is stable for a year by actual test, can be easily prepared. Each first two yards of the hog's gut yields from 75 to 125 doses of secretin.

1. Three hundred grams of the salt precipitate "A" are suspended in 1500 cc. of 95 per cent alcohol. This results in a concentration of alcohol varying from 70 to 85 per cent depending on the amount of water present in "A." This does not disturb the procedure with the exception that the lower the concentration of alcohol the more cholecystokinin will go into solution. The suspension is filtered and the residue on the filter paper is again extracted with a second 1500 cc. volume of 95 per cent alcohol. This is filtered and the two alcoholic extracts are combined. The insoluble residue is retained and called "S III," because it contains 75 per cent of the cholecystokinin and some secretin (usually 25 to 50 per cent). If further extracts of "S III" with the 80 per cent alcohol are made, very little secretin is removed. This additional extraction is not economical (Ivy, Kloster, Lueth and Drewyer, 1929).

2. The combined alcoholic extracts are evaporated to dryness on the water bath with a current of air, adding water occasionally. This addition of water is important since if it is not added, loss of activity results. We add about 750 cc. of water during the evaporation of 3000 cc. of the alcoholic extract.

3. The residue from the evaporation of the alcohol contains a secretin soluble in water and another soluble in 0.4 per cent HCl. The water soluble secretin we call "S I," and the acid soluble, "S II."

The residue is suspended in 2000 cc. of water and allowed to stand 10 or 12 hours. Then NaOH solution is added slowly until the pH is somewhere between 5.3 and 5.7 (quinhydrone electrode). When the pH of the solution has reached 5.0, it is necessary to be careful in adding alkali because only enough to cause maximum flocculation is desirable. The exact pH at which this occurs varies for different batches, but is within the above range for all batches. If the proper care is not exercised at this point, the solution filters neither well nor clear, and a high dosage figure is obtained. If this procedure is correctly performed, the 2000 cc. of filtrate will filter clear in 2 or 3 hours. Secretin is present in both the clear filtrate ("S I") and the precipitate ("S II").

4. The clear filtrate is precipitated by adding sufficient 20 per cent trichloracetic acid solution to give a 5 per cent solution of trichloracetic acid. This is allowed to stand from 4 to 12 hours in the ice-box and then the precipitate is collected by centrifugalizing. The supernatant liquid is not always clear, although the secretin is thrown out readily. The precipitate is washed with absolute anhydrous acetone (aldehyde free) and ether. This precipitate is called "S I pH 1802."

5. The precipitate from isoelectric precipitation of the suspension in step 3, which is on the filter paper, is suspended in 1500 cc. of 0.4 per cent HCl and filtered, using a Buchner funnel and a 2 inch filter paper pad under

TABLE 1

Experiments showing dosage recovery

Each experiment is from an original weight of 300 grams of "A," the NaCl precipitate

EXPERIMENT NUMBER	"S I pH 1802"			"S II pH 1802"		
	Total weight	Dose	Total number of doses recovered	Total weight	Dose	Total number of doses recovered
	mgm.	*mgm.*		*mgm.*	*mgm.*	
1	295	0.6	491	235	0.5	470
2	340	1.5	226	895	0.7	1,278
3	Failed to dry			800	1.5	533
4	330	0.4	660	645	1.0	640
5	220	0.5	440	205	0.3	683
6	180	0.5	360	270	0.5	540
7	330	1.0	330	Failed to dry		
8	118	0.5	236	305	0.5	610
9	285	0.6	441	255	0.4	637
10	450	1.0	450	270	0.5	540
11	350	0.6	583	545	0.7	726
12	420	0.4	1,050	275	0.5	550
	Average = 0.7 mgm.			Average = 0.64 mgm.		

slight suction, until clear or almost so. This filtrate is precipitated with trichloracetic acid at a concentration of 5 per cent. The precipitate is collected by centrifugalizing and is washed with acetone and ether. We call this "S II pH 1802."

For some unknown reason, the precipitates in steps 4 and 5 occasionally fail to dry with the acetone and ether treatment. Out of the 24 trials shown in table 1 this occurred twice.

Method of assay: In assaying our secretin preparations we have used dogs anesthetized with 0.2 gram of sodium barbital (Abbott's) per kilo given by stomach tube, which was reënforced with ether during the operative procedure. We have used dogs varying in weight from 14 to 20 kilos.

For some unknown reason, dogs vary widely in their pancreatic response to secretin, which is at present beyond our control. The weight of the dog is a factor, but is not the chief factor. We have not selected our animals in determining the doses given in table 1. Our impression from experiments on more than 300 dogs is that the amount of juice obtained from an injection is more variable than the threshold dose in different dogs. If the dog is showing a good spontaneous secretion, one obtains a greater amount of juice with the same dose than if there is no spontaneous secretion. We have kept no data to prove this impression, but the problem is being investigated in our laboratory by Miss Hinrichsen. We have arbitrarily adopted the following definition of a threshold dose of secretin: a *threshold dose of secretin* is that amount of dried material in solution which when injected intravenously will cause a 10 drop (0.4 cc.) increase in the rate of flow of pancreatic juice within a ten minute period following the time of injection as compared with the preceding ten minute period.

Vaso-dilatin: There is no vaso-dilatin in either of these preparations even when ten or twenty threshold doses are injected, the "dilatin" being removed by the salt, trichloracetic acid and isoelectric precipitation procedures.

Results obtained with the method: The results of twelve experiments on six different batches of the "A," or salt precipitate in which complete records were kept of the recovery are shown in table 1. The dosage on the different batches of "A" precipitate varied from 75 to 125 mgm. If the total amount of secretin were recovered in "S I" and "S II," the recovery should have totaled from 2400 to 4000 doses. It will be noted from the table that the recovery varied from 846 to 1600 doses, or from thirty to fifty per cent. The chief loss occurs in the extraction with 95 per cent alcohol and cannot be obviated economically by extraction with 80 per cent alcohol after the first extraction with the 95 per cent alcohol.

The milligram dosage, it will be noted, varies from 0.3 mgm. to 1.5 mgm. for both "S I" and "S II." The average dosage per dog for "S I" was 0.7 mgm. and for "S II" 0.64 mgm. The smallest per kilo dosage obtained was 0.01 mgm. and the largest 0.10 mgm. We have occasionally obtained from 5 to 10 cc. of juice with a dosage of 1.0 mgm. per dog, or 0.05 mgm. per kilo. "S I" contains 1 dose of cholecystokinin for 7 to 10 doses of secretin, but "S II" contains no cholecystokinin when as many as 15 doses of secretin are given.

Stability: Our product is destroyed by boiling for 10 minutes with 10 per cent HCl. Boiling a solution when slightly alkaline with NaOH or $Ba(OH)_2$ destroys the activity. It is not destroyed by warm glacial acetic acid. It is not destroyed by boiling in water for one hour. It must be kept in a solution as acid as pH 3.0 in order to retain its activity in the ice-box for periods longer than three weeks.

Solubility: Secretin is not soluble in ether, absolute acetone, chloroform, benzol, alkaline amyl alcohol, ethyl acetate, amyl acetate. Small quantities may be extracted with neutral iso-butyl alcohol. It is partially soluble in absolute alcohol and methyl alcohol (plus 99 per cent).

Heavy metal precipitants and absorbents: Numerous heavy metal precipitants and absorbents have been used but have been found to be of no value in that they either fail to produce appreciable "purification" or destroy or lead to an unrecoverable loss of the activity.

Analysis: We have done a partial analysis of "S I" and "S II," which, of course, we do not believe to be a pure product, but only a concentrate. The results on both "S I" and "S II" are as follows: no phosphorus; no chloride; sulphur, positive (cystine or cysteine); xanthoproteic, negative; Millon, negative; Hopkins-Cole, negative; Pauly, slightly positive; biuret, slightly positive (bluish-red); Molish, negative; Pettenkofer, slightly positive after 5 minutes. Ten milligrams of total solid yielded 0.750 mgm. of total nitrogen. Ash is 0.8 per cent in which iron is present. Heller's ring test is slightly positive after 5 minutes, some preparations being negative.

Mellanby (1928) on analyzing his product obtained a faint sulphur reaction and a well-marked phosphorus test. We obtained no test for phosphorus and a well-marked test for sulphur. We differ also on the xanthoproteic and Millon's reactions, ours being negative and his positive.

SUMMARY

A method for the preparation of a secretin concentrate is described. It has proven to be reliable in our hands and yields a product which is active in doses of one milligram or less in the anesthetized dog.

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MULTIPLE OVARIES AND THE OESTROUS CYCLE IN THE WHITE RAT

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Received for publication June 28, 1930

This problem was undertaken to determine what changes would occur in the oestrous cycle of the albino rat if it were supplied with functioning ovaries in addition to the two it normally possesses. A number of workers have shown that ovaries may be transplanted from one animal to another of the same species successfully (see Steinach, 1912; Marshall, 1922; Long and Evans, 1922; Lipschütz, 1924; and Pettinari, 1926).

METHOD. Twenty-two virgin female rats ranging in ages from 98 to 106 days were used in this experiment. Each of these animals was weighed and placed in a revolving cage for recording its daily activity.

The animals were tested each day by the vaginal smear method as followed by Long and Evans (1922) for five weeks, to determine the length of their oestrous cycles. Following this period the rats underwent an operation for ovarian transplantation. Two normal females of about the same age as the hosts were used in each case as sources for the additional ovaries. These animals had been tested before the operation by vaginal smears to determine what stage of the oestrous cycle they were in. Only rats which were in a stage different from that of the experimental animals were used. The three animals were anesthetized and a median dorsal incision made in each. The ovarian arteries in the source rats were ligated and the ovaries removed. One ovary from a source animal was planted into a kidney of the experimental rat, while an ovary from the second donor was sutured into the muscles of the dorsal abdominal wall. The wound of the experimental animal was closed and the rat returned to the activity cage; daily vaginal tests were taken and the activity recorded.

RESULTS. *Oestrous cycles:* The five weeks before ovarian transplantation served as a control period. During this time the cycles occurred normally as is shown in table 1. Numbers 210 and 223 showed longer cycles than the others—from 6 to 11 days averaging 7.8 days. The periods of the remainder occurred at intervals of 2 to 9 days with an average of 4.2 days.

Following the ovarian transplantation almost an immediate effect was perceived as the cycles became irregular and approximately one-half their normal length in 19 of the 22 animals, the average being 2.2 days, a de-

crease of 48 per cent. The smears in a two-day period often showed cells typical of every stage in a cycle.

TABLE 1

Length of cycles in days

NUMBER OF ANIMAL	BEFORE OPERATION	AVERAGE	AFTER OPERATION	AVERAGE
200	4, 4, 4, 4, 6	4.4	2, 2, 1, 1, 4, 2, 1, 2, 3, 2, 3, 1, 1, 3, 1, 2, 2, 3, 2, 3, 2	2.0
201	3, 5, 3, 4, 3, 4, 5	3.9	3, 3, 2, 2, 1, 3, 2, 1, 3, 2, 2, 3, 2, 3, 2, 3	2.3
202	2, 5, 5, 5, 4, 5	4.3	1, 6, 2, 2, 2, 2, 2, 2, 1, 1, 3, 3, 4, 1, 2, 3, 2, 2, 1, 2, 2	2.7
203	3, 3, 3, 5, 4, 4, 5	3.9	2, 1, 2, 1, 4, 1, 1, 3, 1, 3, 2, 3, 1, 2, 2, 1, 2, 1, 2, 3, 2, 2, 1	2.4
204	2, 7, 5, 3, 5, 5	4.5	1, 4, 3, 2, 4, 3, 2, 2, 2, 3, 3, 1, 2, 3, 2 3, 1	2.4
205	2, 5, 5, 6, 4, 5	4.5	1, 3, 1, 3, 2, 2, 2, 3, 2, 2, 1, 4, 2, 2, 2, 3, 2, 1, 2, 3	2.3
206	3, 4, 3, 4, 4, 5, 4, 6	4.1	2, 1, 5, 1, 2, 4, 2, 3, 1, 1, 2, 2, 3, 2, 2, 1, 2, 1, 2, 2, 2, 2, 3	2.2
207	6, 3, 4, 5, 4, 5	4.5	3, 7, 4, 5, 3, 5, 5, 3, 5, 5	4.2
208	4, 4, 3, 4, 4, 5, 5	4.1	1, 3, 1, 2, 1, 2, 2, 1, 3, 1, 2, 1, 2, 2, 1, 2, 1, 2, 3, 1, 2, 2, 2, 2	1.8
209	5, 3, 3, 4, 3, 4, 4, 4, 4	3.8	1, 4, 2, 1, 1, 1, 2, 1, 3, 1, 1, 2, 2, 2, 2, 3, 2, 1, 3, 2, 2, 1, 2, 2, 3	1.9
210	11, 7, 7, 7	8.0	8, 8, 6, 4, 6, 4, 6	6.0
212	6, 4, 5, 5, 4, 5	4.8	1, 2, 3, 2, 3, 2, 2, 1, 2, 2, 5, 2, 3, 3, 2, 1, 3, 1, 2	2.0
213	2, 5, 5, 4, 6, 4, 6, 5	4.8	1, 2, 2, 1, 3, 1, 4, 1, 1, 3, 2, 3, 3, 1, 2, 1, 2, 2, 2, 2, 2	2.0
214	7, 4, 3, 5, 5, 4, 5	4.9	3, 2, 2, 1, 3, 1, 1, 2, 1, 3, 2, 2, 3, 2, 3, 2, 2, 1, 1	2.0
215	3, 4, 5, 3, 5, 4, 5, 6	4.4	3, 4, 1, 2, 2, 3, 3, 3, 1, 2, 1, 3, 3, 2, 2, 1, 2	2.2
216	7, 9, 6, 7, 5	6.8	4, 4, 1, 3, 3, 2, 2, 3, 3, 2, 2, 2, 2, 2, 2, 2	2.4
217	6, 4, 6, 5, 6, 6	5.5	2, 3, 2, 3, 1, 1, 3, 2, 1, 3, 2, 3, 1, 2, 2, 2, 2, 1, 2, 1	2.0
218	7, 3, 4, 5, 5, 6, 6	5.1	4, 4, 4, 4, 2, 2, 3, 3, 2, 2, 1, 2, 2, 2	2.8
219	4, 4, 3, 5, 4, 5, 7, 4, 5	4.6	3, 3, 1, 3, 2, 2, 3, 2, 3, 3, 2, 2, 2, 2, 2, 2, 1, 2, 1	2.1
220	6, 3, 6, 4, 6, 7, 6, 3	5.1	3, 3, 2, 2, 2, 3, 2, 2, 3, 2, 2, 2, 3, 2, 3, 1, 3, 2	2.5
222	4, 5, 5, 5, 7, 4, 7	5.2	2, 5, 4, 3, 2, 2, 3, 3, 3, 2, 2, 1, 3, 2, 1, 2	2.5
223	9, 9, 6, 7, 7	7.6	5, 4, 4, 5, 6, 5, 4, 3	4.5

Number 210 dropped from 8 to 6 days, a decrease of 25 per cent and number 223 from 7.6 to 4.5 days, a decrease of 40 per cent. Number 207 showed no noticeable diminution in length of periods.

Voluntary activity: The average daily activity of the rats amounted to 710 revolutions before the transplants were made and 818 for the period afterwards, which is an increase of 15 per cent. In general the high peaks of activity came about every five days after the operation as well as before, there being no definite relation between the rhythm of the spontaneous activity and the extra oestrous cycles.

At the termination of the foregoing experiments we attempted to produce pseudo-pregnancy by the method of Long and Evans (1922) in the 19 rats which had exhibited markedly shortened oestrous cycles. They were then from 240 to 250 days old. By means of insertion of a small glass rod into the vagina, pseudo-pregnancy was induced in 10 of the animals. This condition lasted 8 to 14 days, averaging 12. Exposure to a vasectomized male resulted in pseudo-pregnancy in 9 of the animals, this condition lasting 9 to 14 days, averaging 13. Seven of the animals gave positive results with both methods, five with one alone, and seven with neither.

Slonaker (1924–1925) maintains that pseudo-pregnancy commonly results from injuries to the uterus, hence great care was taken with the manipulation of the rod; also if the female objected to the male, he was at once removed. We believe that in neither of the two methods employed was injury to the uterus involved.

Histological study: When the animals were about 290 days old they were sacrificed and a histological study made of the grafts. Active ovarian grafts were found in 14 of the 22 animals. In the others there was no functioning ovarian tissue on the kidney but through an oversight on the part of the junior author (J.L.F.), no sections were made of the muscle transplant in these animals.

The physiological effects of the transplanted ovaries occurred in a remarkably high percentage of our rats.

SUMMARY

1. Multiple ovarian transplants in normal female rats nearly doubled the number of oestrous cycles as demonstrated by the vaginal smear method.

2. The voluntary activity of these rats was not markedly increased after the operation.

3. Pseudo-pregnancy was induced in ten of the nineteen animals by the glass rod stimulation of Long and Evans. Exposure to a vasectomized male produced this condition in nine of these rats.

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THE HORMONAL CONTROL OF LACTATION

I. Non-Effect of the Corpus Luteum

II. Positive Action of Extracts of the Hypophysis

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Received for publication July 7, 1930

I. During the past quarter century a great deal of reflection and experiment has been expended upon the problem of lactation. Since the mammary gland normally undergoes enlargement and histological change preparatory to the secretion of milk during pregnancy only, the cause of these modifications has seemed to lie in some effect exerted upon the organism by one or another of the three new structures which appear in the pregnant animal, namely, the fetus, the placenta, and the corpus luteum of gestation. Halban (1905), by analysis of clinical case-reports, arrived at the conclusion that the placenta or at least the chorionic ectoderm is the agent responsible for the proliferation of the mammary gland during pregnancy and for the secretion of milk from the proliferated gland.

In 1906 Starling and Lane-Claypon published the result of a direct experimental attack on the problem by use of filtered aqueous extracts of ovaries, placentae, uterine tissue, and fetuses. The extracts of fetuses appeared to give rise to some growth of the mammary glands, but the effect was far less than that seen during a normal pregnancy. As Starling afterward realized, the results were doubtful because the animals were not spayed, and therefore the possibility of effects due to ovulation was not ruled out. Confirmatory studies by Biedl and Konigstein (1910) achieved scarcely more definite results, while Frank and Unger (1911) failed to corroborate the work. Experiments with placental extracts, such as those of Aschner and Grigoriu (1911) and Fellner (1913), yielded at best only slight proliferation of the mammary gland.

Another line of experimentation seemed to point to the corpus luteum as a causative factor in the hypertrophy of the mammary gland. Ancel and Bouin (1911) found a marked enlargement of the mammary gland, equalling that seen at mid-pregnancy, at the 14th day of "pseudo-pregnancy" produced by mating female rabbits to vasectomized bucks. These authors supposed, therefore, that the first stage of growth of the mammary gland in pregnancy is induced by a hormone of the corpus luteum, and that

there is a second stage, characterized by continued growth and by the appearance of milk in the ducts, which is due to some other factor. Similar observations of growth of the mammary gland during the period of activity of the corpus luteum, made upon a number of species by various workers, have been well reviewed by Parkes in his recent book (1929a) and need not be detailed here.

Hammond (1917) pointed out that in the rabbit, contrary to some previous observers, the corpora lutea persist throughout pregnancy. When the fetuses were removed (by surgical intervention) from pregnant rabbits at the 13th to the 15th day, there was an arrest of development of the mammary gland and an immediate appearance of milk in the ducts. From these and other considerations he supposed that in pregnancy the corpora lutea are made to persist by an influence arising from the fetuses, and that the persisting corpora lutea in turn induce continued growth of the mammary gland. Whenever growth of the gland has reached a sufficient stage (as, for example, in the rabbit after the 12th day of pregnancy) removal of the stimulating influence causes the secretion of milk. This view seemed to be confirmed by experiments on milch-goats by Drummond-Robinson and Asdell (1926); see also Asdell (1925). On the other hand, observations and experiments by Loeb and Hesselberg (1917) gave very inconclusive results as to a possible direct relationship between the corpus luteum and proliferation of the mammary gland.

Meanwhile it has been established by a series of reports beginning with Fellner (1913) and including among others the work of Herrmann (1915), Vintemberger (1925), Hartman, Dupré, and E. Allen (1926), E. Allen (1927), and Laqueur, Borchardt, Dingemanse and de Jongh (1928), that injections of oestrin (ovarian follicle hormone), in the purified form or as crude preparations of follicle fluid, placenta, etc., produce in immature animals certain definite effects on the mammary glands. The mammillae become enlarged and there is a proliferation of the few primary alveoli which compose the infantile gland, until in the rabbit, for example, the gland-tree may reach one or two centimeters in diameter. Since similar changes occur naturally at the onset of sexual maturity, it may be assumed that this stage of mammary gland development (so-called pubertal development) is controlled by the ovary. Possibly all the positive results obtained by workers with various extracts of placentae, fetuses, etc., were actually due to the presence of oestrin in the extracts.

None of these workers who have attempted direct stimulation with extracts or implantations of placentae, fetuses, corpora lutea or other ovarian tissues, have been able to produce convincing evidence of extensive proliferation resembling that seen in pregnancy, or of active secretion of milk in quantity. Their best results have been in all cases so far short of the normal conditions found in pregnancy, that v. Pfaundler, reviewing

the subject in 1926 in the *Handbuch der normalen und pathologischen Physiologie*, expressed skepticism as to all previous work.

Negative results are, however, not conclusive in investigation of this sort; there is always hope that a fortunate discovery may be made with regard to an extract or other tissue preparation. Of late, conjecture and experiment have been devoted chiefly to the corpus luteum, especially along the line suggested by Hammond's work, mentioned above. His hypothesis could obviously be tested experimentally if some means could be found to prolong the life of corpora lutea in non-pregnant animals through a period equal to that of normal gestation in the same species. Such a means was found by Parkes (1929b) in an ingenious application of the discovery of H. M. Evans (1924) that alkaline extracts of the anterior lobe of the hypophysis induce in female animals luteinization of Graafian follicles and also cause persistence of pre-existent corpora lutea. The mode of experimentation found most satisfactory by Parkes was to produce pseudopregnancy in the does by mating with a vasectomized buck and then after ten to fourteen days to prevent retrogression of the corpora lutea by daily administration of the hypophysis extract. In these experiments growth of the mammary gland went on far beyond that normally found in pseudo-pregnancy. This striking effect was ascribed by Parkes to an endocrine action of the corpora lutea which had been made to persist by the administration of the hypophysis extract.

At the time when these experiments of Parkes were published we were eagerly watching, in this laboratory, to see what effect might be produced upon the mammary glands of our rabbits by administration of the corpus luteum extract ("progestin") prepared by W. M. Allen and the present author, as described in a series of recent papers cited in the appended bibliography under the names of W. M. Allen and Corner. As described in our joint paper of 1930, it has been possible to carry on through pregnancy, and to parturition at or near full term, a number of does which were deprived of both ovaries at about the eighteenth hour of pregnancy. In these animals the mammary glands underwent typical and complete hypertrophy as in a normal pregnancy, and milk was present in the ducts in large quantity. If these functional changes in the mammary gland were actually due directly to the corpus luteum extract, it should be possible to obtain the same effects in spayed non-pregnant does by equal doses of equally potent extracts. This has been put to experimental test. From a number of experiments all pointing in the same direction I select the following two as at first sight conclusive. I am indebted to Mr. Willard M. Allen for much coöperation in these experiments with the corpus luteum extracts.

Rabbit X-136 was one of two does which were mated on May 16, 1929, and spayed on May 17; at operation both were found to have ovulated. Both were given equal doses of the same corpus luteum extracts, 0.5 cc. daily for thirty-two days. On June

18, the other animal (X-137) gave birth to five young; her mammary glands were fully proliferated and were laden with milk as in normal pregnancy at term. X-136 either did not become pregnant or else resorbed her embryos quite early, for an exploratory laparotomy on May 29 revealed that the uterus was empty. On the thirty-second day of the experiment autopsy showed no significant alteration of the mammary glands and no milk present.

Rabbit X-213, born in our colony in September, 1928, was never mated but was kept with other females until about two weeks prior to May 20, 1929. On that day exploration showed that the ovaries contained large fully developed corpora lutea, the uterus was fully proliferated, and the mammary glands were spread out to a diameter of about 3 cm. Both ovaries were removed. This rabbit was then given 28 daily doses of the same extracts (and in the same quantity) which were being given to the two animals (X-136 and X-137) mentioned in the preceding protocol. Autopsy on June 18 showed that the extracts which had carried X-137 through the full period of gestation, had not had the slightest effect upon the mammary glands of X-213, which had in fact retrogressed in size during the experiment; and this in spite of the fact that the animal had been subjected to the action of her own corpora lutea for about a fortnight before the experiment proper was instituted.

In spite of their clear-cut results these experiments may not be conclusive. It is possible, for example, that there is an effect of the corpus luteum upon the mammary gland, but that this requires simultaneous action of oestrin.[1] The non-pregnant spayed rabbits have no oestrin at all, except the small amount (ca. 0.5 rat unit daily) carried in our crude corpus luteum extracts, while the spayed rabbits kept pregnant with progestin possibly receive oestrin from the placentae.

A number of other experiments in which corpus luteum hormone was administered to spayed rabbits for a period equalling or approaching the usual term of pregnancy, without causing significant growth of the mammary gland or lactation, are not reported here because they are, like the two experiments above, not quite free from possible objection on one ground or another. Much time and expense will be required to obtain a series which will meet our own standards of proof. Meanwhile one cannot fail to be influenced tentatively by the fact that in these special experiments, as well as in many scores of tests conducted by Mr. Allen and myself in which the extracts have been administered to non-pregnant rabbits over periods of five to nineteen days, no evidence has been seen of significant changes in the mammary glands or of the appearance of milk.

Another and quite different way of trying the effect of prolonged action of the corpus luteum, which does not involve depriving the animal of her own ovaries, has been provided by the studies of Mr. J. J. Jares (1930), a student in this laboratory, to whom I am indebted for collaboration in the two experiments next to be quoted. Following up Friedman's observa-

[1] Hisaw (1929) and W. M. Allen (1930) have shown that immediately previous action of oestrin is necessary as a preparation for the known effects of the corpus luteum hormones on the symphysis pubis and the endometrium.

tion (1929) that ovulation may be induced in rabbits by intravenous injection of urine from pregnant women, Jares discovered that the presence of recent corpora lutea does not prevent the induction of a second ovulation by means of an intravenous injection of 10 cc. of urine, so that one crop of corpora lutea after another may be produced at intervals of a few days. The rabbit may thus be kept under the continuous influence of her own corpora lutea by repeating the intravenous injection of pregnant urine every ten days. The protocols of two experiments done in this way are given:

Rabbit X-258, born August 1929; kept in isolation since December 1929; operation (ether) February 13, 1930; removal of one mammary gland and exploration to make sure ovaries were mature. Ovaries found mature; no corpora lutea present. The excised control mammary gland was less than 1 cm. in diameter.

Immediately after operation the animal was given, by injection into ear vein, 5 cc. of unfiltered urine from a patient about 7 months pregnant.

February 24, exploration through left flank showed three large corpora lutea in the left ovary. Animal given 10 cc. urine from a patient at full term.

March 6, exploration through right flank showed numerous corpora lutea in the right ovary. Animal given 10 cc. urine from a patient at full term.

March 18, (33rd day of experiment) exploration of abdominal wall showed that the lower mammary glands were in the same state as at the beginning. They were not thickened in the least and alveoli did not extend more than 1 cm. from the mammilla.

No milk or fluid was expressible from the glands at any time during this part of the experiment. Results of subsequent treatment will be given in part II of this paper, p. 52 below.

Rabbit X-259. This rabbit was a litter mate of X-258 (preceding protocol) and the procedure and result were identical in every respect up to March 18 (33rd day of the experiment) when the procedure was changed. No milk or fluid was formed and the glands did not advance beyond the state in which they were found at the beginning.

The outcome of these experiments indicates that the corpus luteum acting alone in the absence of a pregnancy does not induce proliferation of the mammary glands or lactation.

It is surprising that we did not obtain in these experiments even a partial proliferation of the mammary gland such as described by Ancel and Bouin (1911) as occurring in pseudo-pregnancy. Such partial proliferation has been confirmed by other workers, and has been seen in other species than the rabbit. It is difficult to imagine any other cause for it than the corpus luteum. We did not see it in our experiments, but since they were mostly continued for thirty to thirty-three days, it is possible that it occurred and faded out before autopsy. At any rate, I do not intend at this time to insist that the corpus luteum has no effect whatever upon the mammary gland, but only that acting alone it does not produce the conditions seen in the latter half of pregnancy. What effect the corpus luteum may exert upon the mammary gland in pseudopregnancy and in the earlier part of

pregnancy must be studied by further experiments. A new view of this question is suggested by the work next to be reported.

II. Reconsidering the experiments reported by Parkes in the light of the negative result with the corpus luteum alone, it seemed very probable that the profound proliferation of the mammary gland and the lactation obtained in his experiments were actually due to the extracts of the anterior lobe of the hypophysis used by him rather than to the corpora lutea themselves. When I put this supposition to the test by treating spayed virgin rabbits with an alkaline extract of the hypophysis, the logical expectation was fulfilled by the production of complete proliferation of the mammary glands with lactation, closely resembling at its maximal state the condition in the last few days of normal gestation.

Before describing my own experiments in detail, it should be said that while they were in progress Stricker and Grueter, working in the laboratory of Professor Bouin at Strasbourg, announced (1928) the production of lactation in rabbits by the use of similar extracts. The nature of the preparations used by Stricker and Grueter was not explained, except by the statement that they were aqueous extracts of the anterior lobe preserved with tricresol. The usual daily dose was so measured that it corresponded to 0.5 to 1.5 gram of the fresh gland tissue. When administered to sexually mature female rabbits in which corpora lutea had been produced by mating to vasectomized males, lactation always began a few days later. If the animals were spayed ten days after the production of corpora lutea in their ovaries by a sterile mating, administration of the extract after removal of the ovaries produced lactation in spite of the absence of the ovaries. Finally, it was found that it is not necessary to have the animal under the recent influence of her own corpora lutea; the only necessary condition for a successful experiment with the hypophysis extract is that the animal must have had corpora lutea in her ovaries at some time in the past few months. The implication is that the corpus luteum exerts a more or less permanent preparatory action upon the mammary gland, which must have been so prepared before the hypophysis can act upon it. Stricker and Grueter did not succeed in producing lactation in rabbits in the strictly virginal condition.[2] In general, therefore, their findings were similar to those of Parkes, and the two investigations differ chiefly in the interpretation placed upon them. Parkes was inclined to consider the result due to the corpora lutea which persisted through the action of the

[2] The reader should be reminded that rabbits, unlike most other mammals, rarely or never ovulate spontaneously, but only after sexual stimulation by males or (sometimes) other females. If kept in strict isolation after sexual maturity is attained, a doe will never have corpora lutea in her ovaries (except possibly in rare and exceptional cases), whereas virgin females of those species which ovulate spontaneously will, of course, have new corpora lutea in each cycle.

hypophysis extracts; Stricker and Grueter supposed it due to the hypophysis extracts acting upon glands sensitized or partially developed by the corpora lutea.

On looking back over the literature dealing with anterior lobe extracts, one finds a few reports of experiments which (as we can now see) indicated an action of such extracts on the mammary gland. The female dogs used in the work of Putnam, Benedict, and Teel (1929) underwent proliferation of the mammary gland and produced milk. Evans and Simpson (1929, two papers) reported hyperplasia of the mammary glands in rats induced by anterior hypophyseal hormones. In all these experiments on dogs and rats the ovaries were left intact, and the effect on the mammary gland was apparently attributed to the action of corpus luteum tissue called forth in the ovaries by the luteinizing action of the hypophysis hormones.

Turning now to my own experiments, the first group of rabbits included seven young adult females, eight to nine months old, all of which were born in our colony. When these experiments were undertaken, I considered it necessary simply to use rabbits which had never been mated. During the course of the experiments, however, there appeared the paper of Stricker and Grueter (1929) with its claim that the presence of corpora lutea in the ovaries at some previous time, even as long ago as six months before the administration of extracts, is necessary to the induction of lactation. This makes it necessary to review briefly the previous history of our seven rabbits in detail.

Two of them (X-225, X-268) had been isolated at the age of four months and were therefore undoubtedly virgins in the strictest sense, having been removed from the company of other rabbits of both sexes before the onset of puberty, which according to Hammond's tables (1925) never occurs before the age of four months and very rarely before six months. One (X-230) was isolated at the age of five months, and two (X-228, X-229) at six months. Two others (X-247, X-248) were not isolated until the ninth month and had been in isolation one month when used in the experiments. It is highly probable, therefore, that two of the animals had never had corpora lutea in their ovaries, and it is likely that three more at least were in the same condition. This statement can, however, be made only as a probability and not as a certainty, because even in the two which are regarded as having been virgins in the strictest sense, it is remotely possible that spontaneous ovulations may have occurred.

The experimental procedure was as follows: Under ether anesthesia the last (caudalmost) right mammary gland was removed with the overlying skin, using care to secure all blood vessels in the subcutaneous fascia. The abdominal cavity was next opened and both ovaries were removed, together with a piece of the left cornu of the uterus, about 1 cm. in length, taken near the middle of the cornu. All blood vessels were ligated with silk.

The abdominal wall was closed with figure-of-eight sutures of silk. It was found advantageous in closing the skin to place two or three tension sutures of heavy silk well back from the skin edge in order to remove strain from the lockstitch of silk used to appose the skin edges. Sutures were removed on the fifth or sixth day. The animals were returned to their isolation cages after the operation and were examined daily thereafter. It was made a rule in these examinations that the upper (headward) right mammillae should remain absolutely untouched and all manipulations for the purpose of obtaining milk from the mammary glands were therefore carried out on the left and particularly the lower left glands. It may be said at once that in none of these experiments has there been evidence that mere milking or other manipulation of the mammary glands is able to cause any change in them.

In this group of seven rabbits the mammary glands excised at the preliminary operation were all found in the state characteristic of young mature rabbits which have never borne young. The gland consisted of a thin layer of ducts and alveoli spread over an area of not more than 1 cm. radius about the mammilla. The fact that growth of the mammary glands to this degree is frequently found in virgin rabbits and has been produced experimentally in spayed rabbits by the use of follicular hormone (Vintemberger, 1925) makes it highly probable that in none of these rabbits had the mammary gland ever been subjected to stimuli arising from pregnancy or from corpora lutea.

The extracts used in these experiments were prepared in the Research Laboratories of Parke, Davis & Co. of Detroit, Mich., by Dr. E. P. Bugbee. I am deeply indebted to the company and to Doctor Bugbee personally for their generous coöperation. Extracts were made in a uniform manner from whole sheeps' hypophyses by a method modified from that of Teel (1926), involving extraction by an alkaline fluid, subsequent neutralization, and removal of suspended particles as far as possible by straining and centrifugation. The extracts were preserved by the use of a small quantity of tricresol. The use of sheeps' hypophyses rather than those of the ox was suggested to Doctor Bugbee by Dr. P. E. Smith of the College of Physicians and Surgeons, Columbia University, on account of the discovery by Doctor Smith that the hypophysis of the sheep contains a higher content of gonad-stimulating hormone than that of the ox. The extracts were administered once daily, subcutaneously. The dose was uniformly 2 cc., representing about 0.5 gram of the whole fresh hypophysis.

The result of the injections in these seven rabbits was uniform and striking. On the fourth day or thereabouts, a single drop of milk could be expressed from the nipple. The next day there was usually more, and by the seventh many drops of milk could be obtained. At the beginning of the second week, thickening of the gland could usually be felt through

the skin on rolling it between the fingers. By the tenth day the milk could usually be expressed in a stream, and the gland could be distinctly seen through the shaven skin as a dense white area spreading laterally over the abdominal wall. These changes seemed to reach their height about the fourteenth day, and thereafter there was in some of the experiments an apparent slight retrogression in the size of the glands and the amount of milk.

At the time of autopsy, from the fifteenth to the eighteenth day, reflection of the skin demonstrated in all cases a general enlargement and thickening of the mammary glands, far greater than is ever seen in rabbits except during the last week of pregnancy. Even in those rabbits of the group which showed the least change the mammary glands had grown until they met and had spread laterally over the abdominal wall, covering the area normally occupied by the mammary glands during pregnancy. The gland had reached the thickness of 0.5 to 1 cm., and the ducts were flooded with milk until they stood out like white streams coursing towards the nipple. The extent of proliferation and filling of the alveoli differed somewhat in the different animals. Those rabbits of this series which reached the most advanced stage of proliferation and lactation could not be distinguished, as far as the mammary glands were concerned, from animals in the last two or three days of normal pregnancy.

There is good reason to assume that this striking effect of the extracts of the whole hypophysis is not due to the presence of oestrin (ovarian follicle hormone) or of progestin (corpus luteum hormone). At the end of the period of injections, fifteen to eighteen days after spaying, the uterus was invariably found to have undergone castrate atrophy. This in itself suggests that the rabbits were not obtaining oestrin in significant amounts in the hypophysis extracts, for as is now well known, the ability of oestrin to prevent castrate atrophy in rabbits gives an exceedingly delicate test of its presence or absence. It seemed remotely possible, however, that oestrin might be present and acting upon the mammary gland while its effect upon the uterus was in some way being counteracted by the process of lactation. To test this point, one of the seven rabbits in question (X-248) was given daily, in addition to the hypophysis extract, 25 rat units of Estrogen (commercial oestrin made by Parke, Davis & Co., to whom I am indebted for a supply of the preparation). This rabbit underwent proliferation of the mammary gland and lactation as usual; at autopsy, 20 days after spaying, the uterus was of normal size and histological state as if in oestrus. This experiment, showing that oestrin when added exerts its usual action upon the uterus in the presence of the hypophysis extracts, strengthens the evidence that no oestrin is present in the hypophysis extracts.

The absence of corpus luteum hormone (progestin) was not quite so simply demonstrated. Owing to the absence of oestrin and the consequent

castrate atrophy of the uterus, any progestin which might be present in the hypophysis extracts would not be able to exert its characteristic action upon the endometrium. The rabbit (X-248) which received Estrogen by injection, however, should have been a good test for the presence of progestin; no progestational proliferation of the endometrium occurred.

As a further test for the possible presence of progestin in the hypophysis extract, 200 cc. of the latter were extracted by shaking five times with cold peroxide-free ether. After separation the ether was removed by distillation in vacuo and the residue of the ether-fraction was taken up in 200 cc. of Mazola (commercial maize oil). This preparation was tested by our standard rabbit test for progestin (Corner and Allen, 1929). Five daily doses of 5 cc. each, representing 5 cc. daily of the original extract, or 2.5 times the dose used to produce lactation, gave a negative result.

These deductions and experiments indicate that the hypophysis extracts do not contain either oestrin or progestin in significant amounts.

A number of incidental observations and experiments may next be considered.

a. One of the seven animals already described (X-228) was subjected to total hysterectomy, as well as to the usual double oöphorectomy, before administration of the extracts. Since lactation occurred as usual, the experiment demonstrates that the uterus is not involved in the reaction.

b. The presence of the ovaries does not alter the effect of the hypophysis extract.

Rabbit X-250, 8 months old, isolated five weeks, was given the hypophysis extract without previous oöphorectomy. At autopsy after 14 days' treatment, the ovaries were extensively luteinized. The mammary glands were extensively proliferated as in pregnancy of the last week, and milk was obtainable in streams.

Rabbit X-258 (see first part of protocol, p. 47 above) was given the hypophysis extract following the negative result of the first 33 days of the experiment. Exploration at this time showed that the mammary glands were still in the usual pubertal condition. They responded in the usual way to the hypophysis extracts, reaching full development in 12 days. Milk appeared on the second day and was expressible in quantity on the tenth day.

Owing to the high content of the gonad-stimulating hormone or hormones, the ovaries, if permitted to remain, are promptly luteinized, and there is no way of trying the effect of the extracts upon animals having normal ovaries without corpus luteum tissue. Perhaps an approach to this was the rabbit (X-248), which received hypophysis extracts together with Estrogen, and which ran the same course as the others with respect to lactation.

c. One rabbit (X-241) spayed ten days before the beginning of injections, showed only a delayed and partial result.

d. The following experiment was done with three different lots of the

extract. A quantity of the extract was shaken five times with peroxide-free ether. After separation the aqueous residue, which contained practically all the solids of the original extracts, was administered to a rabbit, beginning on the day when it was spayed, as in the first group of experiments. Four rabbits were studied; two of them proved negative as to lactation, and the other two showed only slight growth of the glands. The aqueous fractions, subsequently tested for gonad-stimulating hormone by injection into immature rats, caused prompt growth of the ovarian follicles and enlargement of the uterus. The ether fraction was entirely inactive in this respect, and as has already been said, contained no progestin. It is very difficult to understand this seeming inactivation of the extracts by merely shaking them with ether, which could scarcely have produced a chemical change. The only obvious physical change was a slight flocculation of the suspended solids of the aqueous fraction, so that they tended slowly to settle out upon standing.

Acting on the hypothesis that the ether might have removed some essential substance, several experiments were tried in which the ether fraction and the aqueous fraction were recombined, or administered simultaneously; but the results were conflicting and unsatisfactory. This point requires further study.

e. Heating of the whole extracts to about 90°C. for one hour completely inactivated them.

f. In the earlier stages of this work a number of failures and partial effects from previously tested extracts led to the supposition that the power of producing changes in the mammary gland declines rapidly and is absent after two weeks. Doctor Bugbee tells me that the extracts retain their potency as regards gonad-stimulating hormone for a very much longer period. He was good enough to try the small remainder of a batch which seemed to have lost potency for the mammary gland, and found it still active as to gonad-stimulation. I have not as yet been able to confirm this apparent instability of the lactation-inducing potency by sufficient animal experimentation, but mention the matter here as a possible safeguard to others who may use similar extracts.

In conclusion, it may be said that these experiments demonstrate the production, by extracts of the sheep's hypophysis, of changes in the mammary glands of rabbits resembling those occurring in pregnancy, and leading to the secretion of milk. This effect does not depend upon the presence of the ovaries, for it can be produced in animals spayed at the beginning of the experiment. Very likely, however, the mammary glands must first be subjected to the influence of oestrin (ovarian follicle hormone) and thus be brought into the full pubertal condition at the time of spaying and the beginning of the injections.

In these experiments positive results were obtained in virgin females

which had almost certainly never ovulated, while in those of Stricker and Grueter it was not possible to obtain the effect unless the rabbits had ovulated (and therefore been subjected to the action of the corpus luteum) at some time in the past few months. This discrepancy cannot be explained at present; whether due to a difference in the extracts, or to some other reason, it should, however, yield to further investigation.

There is one significant difference between the state of mammary activity brought about in these experiments and that seen in normal pregnancy. In pregnancy the glands first proliferate without secreting milk, and then toward the end of gestation (as in the rabbit) or after parturition (as in the human) secretion of milk begins. In these experiments the secretion of milk begins as soon as the first growth, and continues *pari passu* with the proliferation. If, as seems likely, the hypophysis is the direct (proximate) cause of lactation in pregnancy, as it is in these experiments, there may be in pregnancy some other mechanism which restrains secretion; or on the other hand, the extracts, undoubtedly very complex chemically, may convey a stimulus not normally released in the pregnant animal until late in gestation.

SUMMARY

1. The administration to non-pregnant spayed rabbits of corpus luteum extract (progestin) did not cause proliferation of the mammary gland beyond the stage normally attained at puberty. The continuous presence of corpora lutea in the ovaries, during a period equal to that of normal gestation (brought about by experimental means) was equally ineffective in altering the mammary gland.

2. Administration of extracts of whole sheep's hypophysis to spayed virgin rabbits caused proliferation of the mammary gland and simultaneous lactation, producing in two weeks a condition scarcely distinguishable from that present at the full term of gestation. Previous preparation by action of the corpus luteum was found not to be necessary to this effect.

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THE INFLUENCE OF THE VAGO-SYMPATHETIC NERVES ON THE VENTRICULO-AORTIC TONUS IN THE TURTLE

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Received for publication June 30, 1930

Tonus waves in the turtle heart have been described by most workers only in the auricle. Rosenzweig's (1) discovery of a sheath of smooth muscle cells underneath the endocardium of the auricle of Emys Europea gave one explanation of auricular tonus waves. Meek (2), in a recent review of the problem, states as a fact that the ventricles do not ordinarily show spontaneous tonus variation and that the ventricular tonus when it does appear takes the form of a certain degree of sustained contraction throughout the diastole rather than distinct rhythm. In cold blooded animals Fano (3) saw ventricular tonus waves only three times in over 100 experiments. Kuliabko (4) reported tonus changes in perfused rabbits' ventricles under the influence of small amounts of veratrine. Pereira (5) reports that the isolated ventricle of the turtle shows occasional tonic oscillations. He distinguishes between auricular and ventricular tonus waves. In the auricular waves the summit and the base line of the graph show wave-like variations, while in the case of the ventricle only the latter is varied, the former remaining horizontal.

Gesell's (6) contention that the ventricular tonus variations in the terrapin's heart are only due to changes in the auricular output which cause tension of the ventricular fibers is disputed by Andrus (7) who was able to demonstrate what he interprets as independent ventricular tonus changes in the terrapin's heart. Shaner (8), in a histological report, states that in the turtle below the atrioventricular valves two masses of smooth muscle pass into the ventricle. Each muscle bundle immediately breaks into many strands, which lie upon the cardiac trabeculae, just beneath the endocardium. The muscle strands subdivide and radiate into the deepest recesses of the ventricular cavity. At the root of the tonus arteriosis the smooth muscle strands pass upwards into the muscular tunic of the great arteries. Shaner thinks that the turtles used in his work were Blanding's Emys Blandingii. He also mentions Eddy's opinion that tonic contraction may be the function of smooth muscle with such a distribution. Snyder and Andrus (9) give further evidence to the effect that tonus waves in the auricle and the ventricle of the terrapin's heart is due to the con-

tractions of the smooth muscle fibers. They have demonstrated that drugs act as poisons on smooth muscle fibers (as papaverin and benzyl alcohol) cause a depression of the tonus waves, while morphine which is said to stimulate smooth muscle fibers causes augmentation.

The evidence of the effects of stimulation of the vago-sympathetic nerves on tonus is still conflicting. Fano (10) claims that vagal stimulation has either no effect or an augmentation of the tonus waves. Bottazzi (11) reports that vagal stimulation causes increased tonus. Bottazzi's finding has been confirmed by Oinuma (12). In regard to tonus changes in the larger blood vessels, these are obviously due to smooth muscle contractions. Roncato (13) reports that the blood vessel movements due to the longitudinal muscle fibers are stronger than those due to the circular fibers, and that stretching intensified or initiated rhythmical contractions in the longitudinal musculature.

METHOD AND RESULTS. The turtles used in this study were of the species Emys Blandingii. The experiments were carried out at room temperature which varied between 24 and 30°C., and drafts were constantly avoided. The hearts were exposed and the vago-sympathetic nerves were separated after the plastron was removed. Isolated heart vagi preparations were made in which the vago-sympathetic nerves were dissected out from high in the neck down to the heart, the pericardium removed and a long strip of the frenulum of the ventricle was left. Into one of the aortae arising from the conus arteriosis a glass cannula was introduced through which Ringer's solution of the following formula was allowed to run. Thus the heart was perfused through its coronary vessels. Ringer's solution used:

	per cent
Sodium chloride	0.7
Potassium chloride	0.03
Calcium chloride	0.025

The frenulum of the ventricle was connected to a light writing lever by means of silk thread and the heart vagi preparation was suspended by the cannula. In this manner the auricles which are in communication with the ventricle by means of narrow muscular tubes produce no mechanical effect on the lever and hence the tonic waves obtained were definitely non-auricular.

To remove any doubt as to the absence of any auricular influences, in some experiments the auricles were cut off entirely and the remaining ventricle kept on exhibiting regular rhythmical tonic waves. In order to estimate the part played by the aorta in the production of the tonic waves, the aortae were separately suspended and tracings obtained.

In some cases the lowest part of the aorta gives rise to regular tonic oscillations which in many cases correspond in time to those recorded by

the ventriculo-aortic preparation. However, the amplitude of the aortic tonus waves is much lower than that of the ventricle. It was therefore concluded that the wall of the aorta which contains smooth muscle fibers (as described by Shaner) plays a part in the production of tonus waves recorded from the ventricle and hence the term ventriculo-aortic tonus waves (fig. 1). The tonus waves were regularly observed as long as the temperature was kept above 24°C. and there was no air draft. On each tonus wave were superimposed 20 to 25 ventricular beats and this ratio remained constant if no change in the environment took place. The aver-

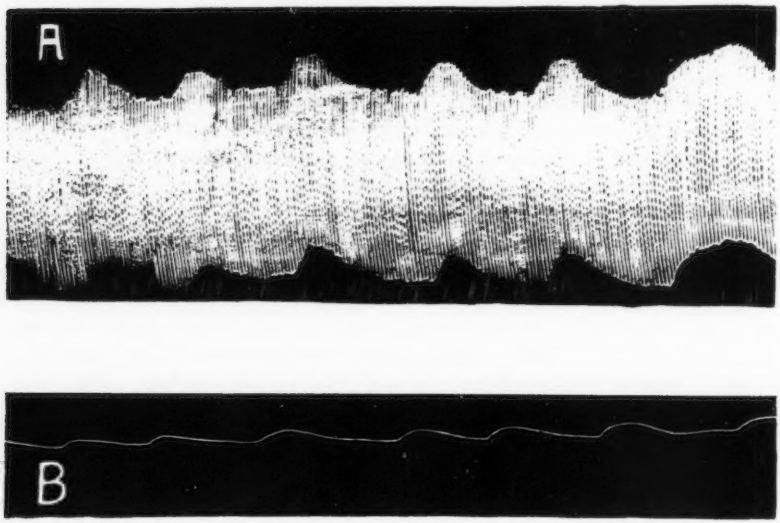

Fig. 1. Showing (A) the ventriculo-aortic tonus waves and (B) the aortic waves of contraction.

age heart rate was 22 per minute and the amplitude of the tonus waves ranged between 3 and 6 mm.

Changes in temperature had distinct effects on the rate, amplitude and regularity of the tonic waves. The effect of cold was tested by introducing the entire set up into an ice box in which the temperature was kept at −2°C. The records showed a gradual decrease in rate and amplitude of the ventricular beats and the tonus waves, although the latter were affected more markedly and disappeared before the ventricular beats. The ratio between the ventricular beats and the tonic wave gradually increased from $\frac{1}{25}$ to $\frac{1}{16}$ and $\frac{1}{6}$ before the tonus waves disappeared (fig. 2).

The effect of heat was studied by enclosing the preparation in a glass

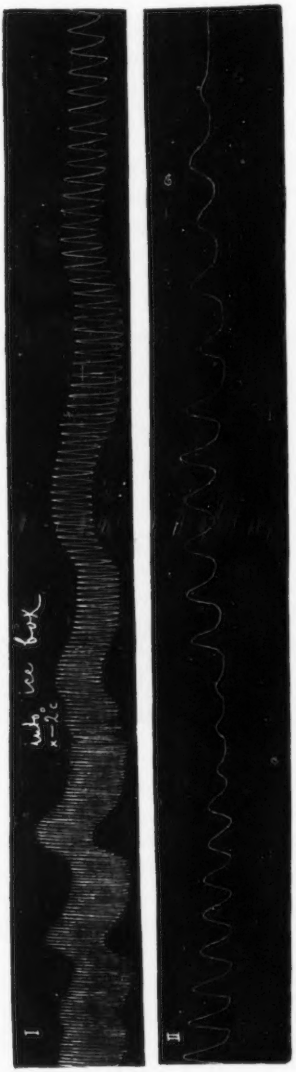

Fig. 2. Showing the influence of cold upon the ventriculo-aortic tonus waves

chamber and heating the chamber. With the rise in temperature in the chamber the tonus waves showed an increased rate and amplitude accompanied by a similar change in the cardiac beats. At the same time the ratio between the cardiac beats and the tonus waves decreased from $\frac{1}{2}$:5 to $\frac{1}{3}$:5 and $\frac{1}{4}$:5. In addition to that, the high temperature disturbed the regular rhythmicity of the waves both in rate and in amplitude, as seen in the tracing (fig. 3).

The effects of pure oxygen or carbon dioxide on the tonus waves were studied by enclosing the heart in a glass chamber through which these gases passed. No distinguishable effects were noted in nearly pure oxygen, while a high concentration of CO_2 depressed ventricular beats and increased the amplitude of the tonus waves.

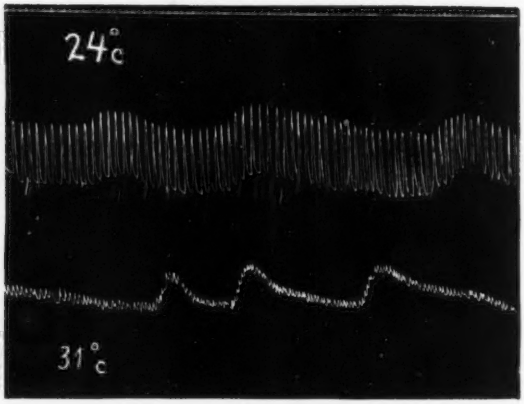

Fig. 3. Showing the effect of elevation of temperature on the ventriculo-aortic tonus waves.

The actions of adrenalin and ergotamine were tried by introducing the solution into the ventricular cavity through the aorta. The solutions used were 1:1,000,000,000 and 1:20,000 respectively. Adrenalin showed in all cases a depressing influence on the tonus waves while ergotamine had no effect on the tonus.

The vago-sympathetic nerves of the preparation were stimulated by the faradic current. On stimulation for short periods of time, ventricular inhibition usually occurred (if the nerves were not injured in making the preparation) but no definite effect on the tonus waves could be demonstrated. The tonus waves seemed to follow their own course in spite of the vago-sympathetic stimulation, as seen in the tracing (fig. 4). On prolonged stimulation of the vago-sympathetic nerves and whenever a ventricular

escape did not take place, it was possible to note the persistence of the tonus waves with rate and amplitude unchanged. In the longest period of inhibition that was recorded (12 min.) 11 distinct tonic waves appeared free from ventricular beats.

Discussion. The observations described above indicate in the turtle the presence of ventriculo-aortic tonus variations which seem to be similar in nature to those described in the auricle. As Rosenzweig's histological description gave support to the non-striated muscle theory of tonus waves in the auricle so does Shaner's report of the presence of a smooth muscle sheath running from the ventricle to the conus arteriosus give the anatomical basis for a similar interpretation of the ventricular tonus.

The part played by the aorta in the production of these waves is small because in most cases no spontaneous movement of the longitudinal musculature of the separated aortae could be detected and wherever the rhythmical movements of the aorta were recorded with and without

Fig. 4. Showing that stimulation of the vago-sympathetic nerve causes no change in the character of the ventriculo-aortic tonus waves.

stretching, they were considerably smaller in amplitude than those obtained in the original preparation and in many cases their rhythm was very different from the original ones. A comparison between the waves obtained during prolonged inhibition and those recorded by the aorta might be of interest because it indicates clearly that the ventricle actually plays an important part in the production of the tonus waves. However, on cutting off the aorta, the pure ventricular waves could hardly be recorded, in most cases they were imperceptible. This peculiar behavior might be due to the fact that the section between the ventricle and the conus arteriosus destroys the anatomical and physiological continuity of the mechanism.

Since stimulation of the vago-sympathetic nerves under the condition of our experiments did not affect the tonus waves in any manner, it seems probable that the mechanism responsible for the production of this tonus variation is independent of the control exerted by the vago-sympathetic nerves on the ordinary ventricular rhythm. This finding agrees to some extent with Fano's observation on the turtle auricle, where vagal stimulation never inhibited auricular tonus waves but left them either uninfluenced or

often increased. In the ventriculo-aortic tonus waves no indication of an increase could be noted. Snyder and Andrus' findings that no change in the auricular tonus waves occurred on the application of atropine to the terrapin's heart, are quite in harmony with our findings on the ventriculo-aortic tonus waves.

The influence of heat and cold on the tonus waves of the ventriculo-aortic preparation is essentially the same as that described by Pereira (14) for the auricular tonus waves, namely, that heat increases the rate and amplitude of tonus waves while cold has the opposite effect. It is worthy of note, however, that in the experiments described in the present paper cold has a stronger depressing effect on the ventriculo-aortic tonus waves than on the ventricular beats, causing the tonus waves to disappear earlier than the cardiac beats. On the other hand, heat acted more favorably on the tonus waves than on the ventricular beats causing a depression of the ventricular beats first. It appears therefore that the tonus mechanism has a wider temperature range than the rapid ventricular rhythm, a fact which might lend further support to the view that the mechanisms producing the ventricular beats and the tonus waves in the ventricle are of a different character.

The stimulating action of CO_2 on the ventricular tonus may be due to a change in pH of the medium, which according to Snyder and Andrus plays an important rôle in the maintenance of tonus waves in the auricles and the ventricles of terrapin's heart.

The effect of adrenalin is similar to that described by Gruber (15) on the sino-auricular musculature.

CONCLUSIONS

1. Independent ventriculo-aortic tonus waves were recorded in the turtle preparation described.

2. The ventriculo-aortic tonus waves are not influenced by stimulation of the vago-sympathetic nerves.

3. Temperature elevation causes an increase of the ventriculo-aortic tonus waves, while cold has the opposite effect. The most favorable temperature for the tonus mechanism is higher than that for the other forms of cardiac rhythm.

4. CO_2 in high concentration stimulates the ventriculo-aortic tonus mechanism. High concentration of oxygen has no effect on the tonus.

5. Adrenalin in concentrations used causes a depression of the tonus mechanism. Ergotamine has no effect.

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RELATIONSHIP BETWEEN VITAMIN C AND SOME PHASES OF REPRODUCTION IN THE GUINEA PIG[1,2]

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Received for publication May 29, 1930

Relatively few studies have been made upon the dietary needs of the guinea pig for reproduction. Papanicoloau and Stockard (1919–1920) discovered that inanition prolonged the dioestrous interval of the ovulation cycle. Ingier (1915) in a series of experiments in which guinea pigs were deprived of vitamin C during different stages of gestation, established that the gravid animal was more susceptible to scurvy than others, that pronounced scurvy might be produced in the fetus as early as the tenth to the fifteenth day after the diet of the mother had been changed, and that frequently the fetus was born prematurely or still-born. These facts were confirmed by Reyher, Walkoff, and Walkoff (1928). Linsay and Medes (1924–1925), (1926) reported that male guinea pigs with mild chronic scurvy did not reproduce and they described extensive histological changes in the germinal epithelium of the testes.

In the course of some experiments conducted at the Pharmacological Laboratory of the Pharmaceutical Society of Great Britain, on the assay of vitamin C, two observations were made concerning the reproduction of guinea pigs, which led to the present investigation. First, females on the Zilva scorbutic diet with various daily doses of orange juice, including doses high enough to prevent scorbutic lesions in the teeth as described by Höjer (1924), (1926), failed to manifest oestrus during 42 days of experiment, and secondly, smears from the epididymis of males, dying of scurvy, contained motile sperm. That the oestrous rhythm is a sensitive indicator of the nutritional state of an animal was pointed out by Papanicoloau and Stockard (1919–1920) in some inanition experiments with the guinea pig, and by Evans and Bishop (1922) in a study of nutritional deficiencies in the rat. When, in the early experiments with the Zilva scorbutic diet, even those females receiving a protective dose (3 cc.) of orange juice

[1] The data in this paper are taken from a dissertation submitted by the author in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Columbia University, 1930.

[2] This work was aided by the Research Grant from the Chemical Foundation to the Department of Biological Chemistry.

failed to exhibit oestrus, it was clearly apparent that the diet must be lacking in some necessary constituent, other than vitamin C. For the present experiments, therefore, a modification of the Sherman (1922) scorbutic diet was chosen.

EXPERIMENTAL. The following diet 2 was used:

	Parts
Equal volumes Pillsbury's bran and quaker oats	580
Merrell Soule skimmed milk powder baked 2 hrs. at 110°C.	300
Milk fat from sweet butter	100
NaCl	10
Cod liver oil (Mead's)	10

(The diet was freshly prepared 2 or 3 times a week)

Supplementary (see table 1) doses of fresh orange juice were administered daily by pipette.

TABLE 1

Length in days of successive oestrous cycles of guinea pigs on the scorbutic diet with decreasing amounts of vitamin C

NUMBER OF GUINEA PIG	STOCK DIET	SCORBUTIC DIET NO. 2				REMARKS
		3 cc. orange juice	½ cc. orange juice	¼ cc. orange juice	⅛ cc. orange juice	
♀ 9	17, 18, 16	31, 16, 15	16, 16, 15	16, 16, 16	Decline	Cured; 3 cc. orange juice
♀ 10	18, 17, 15 19, 16, 14	14, 15, 18	18, 14 19, 14	15, 16 Decline		Severe scurvy at autopsy
♀ 26	20, 15, 17	15, 15, 15	15, 15, 15	15, 15, 15	15 Decline	Severe scurvy at autopsy

Guinea pigs weighing 250 to 300 grams were secured from a dealer and were bedded on wood shavings. Their daily weight as well as food consumption was recorded. The females were examined daily for the vaginal membrane of dioestrus, described by Stockard and Papanicoloau (1919) and the occurrence of oestrus was confirmed by a microscopic study of the smears obtained when the membrane was found to be ruptured (1918).

Effect of vitamin C deprivation upon oestrus rhythm. Young control guinea pigs kept on the stock diet of oats, bran, hay, and greens, had more irregular cycles than those described by Stockard and Papanicoloau (1918), who reported a mean length of 15.74 days with variations no greater than from 15 to 17 days. The mean length of 70 cycles, occurring in 15 guinea pigs under the conditions of this laboratory, was found to be 17.43 days with a probable error of the mean of ± 0.24 day and a standard deviation of ± 3.02 days with a probable error of the standard deviation of ± 0.17 day.

As the animals grew older, however, the cycles more nearly approximated the description of Stockard and Papanicoloau; for when the same guinea pigs were studied after they had had one litter, the mean length of 30 cycles was 16.00 days with a probable error of the mean of ± 0.10 day, and a standard deviation of ± 0.88 day with a probable error of the standard deviation of ± 0.07 day. The irregularity of the earlier cycles may have been due to the difficulty with which guinea pigs adjust themselves to strange environment and new dietary regime.

Three guinea pigs, which had had a normal oestrous rhythm upon the stock diet, were transferred to the scorbutic diet 2 with a daily protective dose (3 cc.) of orange juice. The regular occurrence of oestrus was soon resumed and maintained for about 150 days, during which the orange juice was fed successively at the levels of 3 cc., $\frac{1}{2}$ cc., and $\frac{1}{4}$ cc. daily for about 50 days on each level. Table 1 shows the regularity of the oestrous rhythm even though the animals were receiving far from enough orange juice to prevent chronic scurvy. Guinea pig ♀ 26 manifested oestrus after the level of orange juice had been reduced to $\frac{1}{8}$ cc., just before the rapid decline in growth, due to scurvy, set in. At this time the animal had painful stiff joints, and 20 days later, at autopsy, there were marked lesions of scurvy, such as general hemorrhages throughout the body, hemorrhages of the knee joints, enlargements of the costochrondral junction of the ribs, and loose molars. The ovaries contained large follicles. Guinea pig ♀ 10 was autopsied 16 days after the last oestrous period and the autopsy record was similar to that of ♀ 26. The third animal of the group, ♀ 9, after 34 days of dioestrus on $\frac{1}{8}$ cc. of orange juice, was cured by the administration of 3 cc. orange juice daily. The oestrous rhythm was reëstablished within 10 days of the rise in growth curve.

Chart 1 illustrates the growth curve of guinea pig ♀ 9, which was typical of the group. The circles indicate the day of oestrus. Oestrus was regularly maintained throughout a period of about 100 days, during which the animal received daily only $\frac{1}{2}$ cc. or less of orange juice, amounts which have been shown to permit characteristic scorbutic changes in the teeth after 14 days (Höjer, 1924).

Loss of weight in guinea pig during oestrus. It is apparent from the chart that while there was considerable fluctuation in the daily weight of the guinea pig, a significant loss usually occurred on the day of oestrus, which was sometimes as great as 40 grams. The loss in weight was not accompanied by any noticeable sign of malaise and was quickly regained. There was no corresponding decrease in the food consumption, such as Slonaker (1924–1925) has observed in the rat.

A loss in weight during oestrus seems to be characteristic for the guinea pig, an observation which was repeatedly confirmed in females on the stock diet, although it did not invariably occur. Rats, on the other hand, did

not change in weight at any particular time of the cycle, when they were weighed several times daily. This phenomenon may account for some

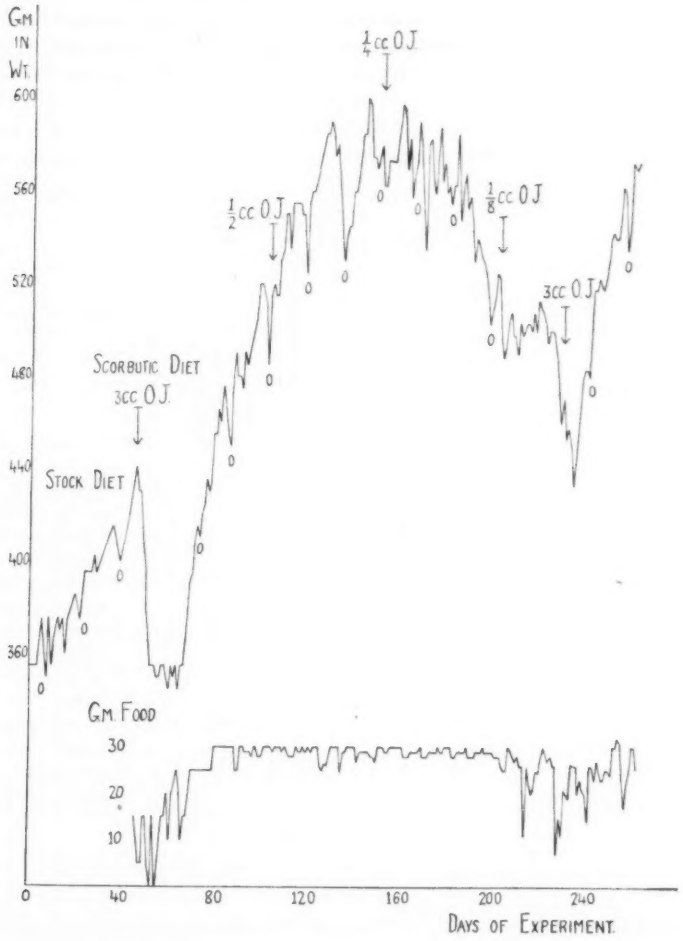

Chart 1. Growth curve, oestrous rhythm, and daily food consumption of guinea pig ♀ 9. Scorbutic diet 2 with different levels of orange juice. Letter *O* indicates oestrus.

of the large fluctuations in weight observable in guinea pigs during vitamin C tests.

Effect of vitamin C deprivation upon the reproduction of the male guinea pig. With regard to the influence of vitamin C deprivation upon the reproductive capability of the male, it was found that smears from the epididymis of twelve guinea pigs dying of chronic scurvy contained normal looking motile sperm. This observation is not in disagreement with the description by Linsay and Medes (1926) of the histological changes in the testis

TABLE 2

Breeding behavior of male guinea pigs on the scorbutic diet with decreasing amounts of vitamin C

NUMBER OF GUINEA PIG	DIET ON WHICH REARED	WEIGHT BEGINNING OF EXPERIMENT	STOCK DIET			SCORBUTIC DIET NO. 2, 3 cc. ORANGE JUICE			SCORBUTIC DIET NO. 2 LESS THAN 3 cc. ORANGE JUICE			REMARKS UPON AUTOPSY
			Days on diet	Number attempted matings	Number litters sired	Days on diet	Number attempted matings	Number litters sired	Days on diet	Number attempted matings	Number litters sired	
		grams										
♂ 1	Stock	350							42	5	0	Severe scurvy, motile sperm
♂ 5	Stock	330							73	3	0	Severe scurvy, motile sperm
♂ 6	Stock	300							87	5	0	Severe scurvy, motile sperm
♂ 46	Stock	580	46	7	6				30	7	0	Severe scurvy, motile sperm
♂ 50	Scorbutic greens	755	21*	10	3	31	14	1	21	10	0	Not autopsied, cured
♂ 34	Scorbutic ½ cc. orange juice	213							227	4	0	Severe scurvy, motile sperm
♂ 36	Scorbutic 3 cc. orange juice	195				195	10	3	60	3	0	Severe scurvy, motile sperm
Total				17	9		24	4		37	0	

* Scorbutic diet with greens.

during scurvy and inanition, for these authors showed that the degeneration was uneven throughout the gland and that normal tubules existed among the disintegrating ones.

As the motility of sperm is no criterion of their normal fertilizing power, some functional tests were initiated. Seven young adult males were deprived gradually of vitamin C during a period of from 1 to 8 months, by giving them scorbutic diet 2 with decreasing levels of orange juice. At frequent intervals, they were mated with normal females in oestrus. When

moribund, they were killed and autopsied, and without exception showed lesions of severe scurvy as well as motile sperm.

The females were maintained on the stock diet and were known to have had a litter either before or after the mating with the experimental animals. Vaginal smears were daily examined for plug and sperm during the mating period of two or three days, but in so few instances were they detected, that the number of litters sired per number of attempted matings was used as the index of breeding function. When 6 control males on the stock diet were bred with normal females, of 31 attempted matings, 24 (or 80 per cent) resulted in litters. The individual records varied considerably.

Table 2 summarizes the results of the breeding experiments with adult males on the scorbutic diet 2 with decreasing amounts of orange juice. The males were not mated more frequently while they were on the stock diet or on the scorbutic diet with a protective dose of orange juice, because of the limited number of females.

It will be seen from the table that not one litter was produced as the result of 37 attempted matings of males on the scorbutic diet with less than the protective dose of orange juice, a confirmation of the work of Linsay and Medes (1924–1925). With two of the same males on a protective dose (3 cc.) of orange juice, the number of litters sired was only 4 out of 24, or 16 per cent of the total number of attempts. While the mating behavior of the animals was not studied in the way described by Avery (1925) and Louttit (1927), it was observed that males on a vitamin C-low diet seemed to retain the reactions of normal males when females were introduced into the cage.

Since the functional tests were negative, it was necessary to resort to artificial insemination in order to determine the fertilizing power of the sperm. Motile sperm taken from the epididymis and testes of ♂ 6, dying of scurvy after 87 days on the vitamin C-low diet, were injected directly into the exposed uterine horns of a female in oestrus.[3] Sixty-nine days later, the female delivered a litter of three normal young. ♂ 34 and ♂ 36, the histories of which are included in table 2, were members of this litter.

One curative experiment was attempted. ♂ 50 of table 2, which had been reared on the scorbutic diet with greens, showed the typical reduction in ability to sire litters when 3 cc. of orange juice were substituted for the greens. During 33 days on a daily dose of ½ cc. orange juice, no litter at all was sired in 10 attempts and the male lost weight and was obviously in poor condition. After the experimental period, greens were given again instead of orange juice. The animal completely recovered and in 6 months was mated with a female in oestrus, when a normal litter resulted.

[3] I wish to express my thanks to Dr. E. T. Engle of the Department of Anatomy for suggesting and performing the operation required for the artificial insemination.

It appears, then, that although males with chronic scurvy have lost the ability to sire litters, the sperm retain their motility and are capable of fertilizing the ovum. The decrease in general vigor is probably responsible for the first condition. The fact that the procreative power of males on the scorbutic diet with sufficient orange juice to insure protection against scurvy is considerably reduced, may indicate that the diet is not complete in all respects.

SUMMARY

1. The oestrous rhythm is ordinarily a sensitive indicator of the nutritional state of an animal. When guinea pigs with normal cycles were gradually deprived of vitamin C, the oestrous rhythm was found to be maintained regularly until the animal began to lose in weight. The cycles continued although the animals were receiving far from enough orange juice to protect them from chronic scurvy.

2. The onset of oestrus in the guinea pig was found to be accompanied by a sudden and temporary loss in weight, amounting sometimes to 8 per cent of the weight of the animal.

3. When males were gradually deprived of vitamin C, they lost their ability to sire litters. Smears from the epididymis of males dying of chronic scurvy contained motile sperm. That such sperm had not lost their fertilizing function was proved by artificial insemination. In a curative experiment, a male recovered the ability to sire litters.

It gives me pleasure to acknowledge my gratitude to Dr. H. T. Clarke and Dr. G. L. Foster for their kind help and encouragement.

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EFFECT OF CONTINUOUS INTRAVENOUS INJECTION OF EPINEPHRINE ON THE CARBOHYDRATE METABOLISM, BASAL METABOLISM AND VASCULAR SYSTEM OF NORMAL MEN

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Received for publication June 30, 1930

In men the subcutaneous injection of 0.5 to 1.0 mgm. of epinephrine is followed by a rise in blood pressure. In the common laboratory animals (dog, cat, rabbit) 0.5 to 1.0 mgm. per kilo, injected subcutaneously, does not produce a rise in blood pressure, though this corresponds to a dose of 35 to 70 mgm. for a man of 70 kilos. There are two possible explanations for this difference. Either epinephrine is absorbed more rapidly from the subcutaneous tissue of man than from that of the animals mentioned or the rate of absorption is the same but the vascular system of man responds to smaller amounts of epinephrine than that of the other species. Starting from this problem an investigation was begun of the sensitivity of the vascular system, the carbohydrate metabolism and the basal metabolism of various species during continuous intravenous injection of epinephrine, the aim being to determine the minimal effective rate of injection. Experiments of this nature on rabbits (1) and on rats (2), have been reported previously. The present paper concerns 17 experiments performed on 9 medical students of the University of Buffalo.[1]

Experimental. The subjects were instructed to come in the morning to the laboratory in a fasting condition. After a rest period of 30 to 50 minutes on a bed the basal values for pulse and respiration rate, blood pressure and body temperature were determined. One or two metabolism periods of 6 to 8 minutes were taken, using the new type of the Benedict-Roth machine and graphic registration. All the subjects were thoroughly accustomed to the procedure. The basal period generally checked within a few per cent with previous determinations of the basal metabolism on the same subject. As a test for leaks a weight was placed on the spirometer bell for two minutes during each test as recommended by Benedict.

[1] The authors take this opportunity to thank Mrs. Hicks and Messrs. Javert, Kavle and Maher, who volunteered as subjects and who participated in the experimental work.

At zero minutes a needle was introduced into a cubital vein, a blood sample was taken without stasis and the infusion of salt solution was started at a rate of 1 cc. per minute for 20 minutes. During the saline injection observations of pulse and respiration rate and blood pressure were made every 5 minutes and the basal metabolism was determined from the 6th to the 12th minute. At the end of the saline injection another blood sample was drawn, a procedure which caused no discomfort to the subject since the needle was already in the vein. Immediately afterwards the injection of epinephrine was started and continued for 30 minutes at a rate of 1 cc. per minute. Pulse and respiration were again counted at regular intervals, blood pressure was determined and two metabolism periods were made, one beginning 6 and the other 22 minutes after the start of the epinephrine administration. A third blood sample was taken at the end of the epinephrine injection and the observations were continued for another half hour. The metabolic rate was again determined 15 and 30 minutes after the end of the epinephrine administration and the experiment terminated by drawing a last blood sample.

The infusion apparatus was extremely simple and was easy to sterilize. It consisted of a short piece of rubber tubing with an adapter for the needle on one end and for a 30 cc. glass syringe on the other end. The syringe which was graduated in 0.5 cc. was fixed by a clamp to a stand and the plunger was pushed in one division every 30 seconds. When it was desired to take a blood sample it was merely necessary to detach the rubber tubing from the needle in the vein, to let a few drops of blood run out and to attach a dry syringe with which to draw blood. The infusion could be resumed afterwards or could be changed from salt solution to epinephrine without the knowledge of the subject.

Blood pressure was determined by the auscultatory method. The mercury manometer—placed in such a way that it could not be seen by the auscultator, was read by another observer at a given signal. Blood pressure was not determined in all experiments because it was feared that the repeated stasis of the fore arm might influence the blood lactic acid values. This fear proved however groundless as was found in subsequent experiments, especially since blood was never drawn from the arm which was subjected to stasis. In other cases the determination of blood pressure was omitted because it interfered with the determination of the basal metabolism. To make up for this 3 experiments were performed in which the measurement of the blood pressure was the main objective. In some of the earlier experiments the preliminary period of saline infusion was omitted and the metabolic rate was not determined. The advantage of the preliminary period is obvious. It allows the subject to get used to the exerimental procedure so that any nervous excitement which might have been present in the beginning has time to wear off. Secondly, the range of

fluctuation of the various factors determined is illustrated under conditions which but for the injection of the hormone are identical to those obtaining during the epinephrine administration.

For the determination of blood sugar the precipitation of Somogyi (3) was used in conjunction with the Hagedorn and Jensen method; this gives "true sugar" values. The same precipitation was not used for lactic acid determinations because a small amount of the lactic acid is carried down by the protein precipitate. The Folin-Wu deproteinization was used instead and after treatment of the filtrate with copper sulfate and lime, lactic acid was determined by means of the Friedemann and Kendall (4) method.

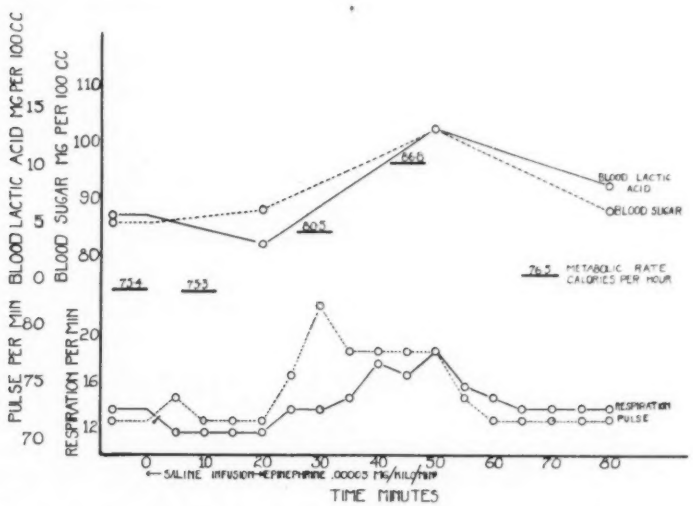

Fig. 1. Metabolic changes produced in a normal man by continuous intravenous injection of epinephrine at a rate of 0.00005 mgm. per kilo per minute.

Three rates of injection of epinephrine were used, namely, 0.0001, 0.00005 and 0.000025 mgm. per kilo per minute. Depending on the weight of the subject, this required dilutions of epinephrine of 1 to 100,000 to 1 to 700,000. The dilutions were made from the 1 to 1000 adrenalin chloride solution of Parke, Davis & Co., using sterile physiological salt solution. The latter was prepared from water distilled first over permanganate and then over barium hydroxide in an all-glass distilling apparatus.

A typical experiment, performed in the manner described above, is illustrated in figure 1. The data of the other experiments are shown in tables 1 to 3, the material being divided into the three main headings indicated in the title. The date of the experiment and the name of the subject make it possible to identify the same experiment in the different tables.

Changes in carbohydrate metabolism. A rate of injection of 0.0001 mgm. per kilo per minute was used in 3 experiments and in each case there occurred a rise in blood sugar and lactic acid (table 1). The average increase in the former amounted to 44 and in the latter to 10 mgm. per 100 cc. Muscular tremor was not observed during the epinephrine administration. The subjects felt a pulsation in the large arteries but otherwise they were free of disagreeable sensations. In the period after the injection the percentage of blood sugar and lactic acid diminished but the basal values were not yet reached 30 minutes after the end of the epinephrine administration.

TABLE 1

Changes in blood sugar and blood lactic acid during constant intravenous injection of epinephrine

DATE	SUBJECT	WEIGHT	BLOOD SUGAR				BLOOD LACTIC ACID				RATE OF INJECTION
			Initial	Salt solution	Epinephrine	30 min. later	Initial	Salt solution	Epinephrine	30 min. later	
		kgm.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm./kilo/ minute*
November 19	Hi	55	80		12)		8.5		14.4		0.0001
November 21	De	88	95		140	129	7.2		23.2	10.8	0.0001
December 9	Sa	60	85	86	123	107	14.1	12.3	22.3	17.0	0.0001
November 26	Gr	62	81		132	109	7.4		11.7	7.8	0.00005
December 3	Hom	75	77		114	101	10.1		14.4	10.9	0.00005
March 4			97	92	132		9.7	10.8	20.2		0.00005
January 16			83	87	100	85	6.4	6.2	7.6	7.1	0.000025
December 11	Hob	76	88	83	103	91	5.6	6.5	13.3	5.2	0.00005
February 11			94	94	120						0.00005
January 22			94	89	103	94	8.0	8.7	8.0	9.1	0.000025
April 22	Ka	91	81	84	121		9.5	9.7	21.2		0.00005
May 13			88	92	119		9.9	8.4	9.8		0.000025
April 15	Ja	75	77	78	96		4.2	5.2	12.7		0.00005
May 15	Ma	62	94	94	106		8.0	5.5	8.5		0.000025

Very uniform results were obtained in 7 experiments in which the rate of injection was 0.00005 mgm. per kilo per minute. During the 20 minutes of injection of salt solution no significant changes were observed in blood sugar and lactic acid, while at the end of the epinephrine administration both were distinctly elevated, the former on an average by 32 and the latter by 8 mgm. per 100 cc. Some of the subjects were unable to indicate with certainty whether salt solution or epinephrine was being injected. In the half-hour period after the injection the blood lactic acid returned to the basal level, the blood sugar remained however slightly above normal. The injection of epinephrine at a rate of 0.000025 mgm. per kilo per minute was

without influence on blood lactic acid but was still effective as far as blood sugar was concerned, as is shown by an average increase of 17 mgm. per 100 cc. for the 4 experiments recorded in table 1.

The blood sugar level of man reacts with greater sensitivity to epinephrine than the blood lactic acid level. This was not found to be the case in rabbits (1). In this species the minimal effective rate of epinephrine administration of 0.00005 mgm. per kilo per minute caused an increase in both blood sugar and lactic acid. The white rat was found to be markedly less sensitive to epinephrine since an intravenous injection rate of 0.0002 mgm. per kilo per minute was required to produce a perceptible rise in blood sugar (2).

TABLE 2

Changes in metabolic rate during continuous intravenous injection of epinephrine

DATE	SUBJECT	AVERAGE BASAL CALORIES	PER CENT OF NORMAL*	SALT SOLUTION	EPINEPHRINE		PER CENT INCREASE OVER BASAL	AFTER EPINEPHRINE		RATE OF INJECTION
					6 minutes	22 minutes		15 minutes	30 minutes	
										mgm./kilo/minute
February 11	Hob	75.4	−1.4	75.3	80.5	86.8	15.4	76.5		0.00005
April 22	Ka	79.1	−4.7	77.2	81.3	85.5	8.1	83.0	76.9	0.00005
May 13		79.8	−2.8	72.4	81.5	82.5	3.4	82.2	80.4	0.000025
March 4	Hom	75.1	−2.4	69.5	80.6	86.0	14.5	68.9	73.4	0.00005
April 15	Ja	76.9	−1.0	77.8		89.7	16.6	83.1	77.2	0.00005
May 15	Ma	60.2	−12.3	60.5	66.9	61.4	2.0	61.0		0.000025

* Aub-DuBois standard.

One hour after the subcutaneous injection of 1 mgm. of epinephrine in men the average increase in blood sugar amounted to 76 mgm. (5). Judging from the experiments in table 1, this is similar to the effects produced by an intravenous injection of epinephrine at a rate of 0.0001 mgm. per kilo per minute. If absorbed at this rate 1 mgm. of epinephrine, injected subcutaneously, would disappear in $2\frac{1}{2}$ hours in a man of 70 kilos.

Subcutaneous injection of 1 mgm. of epinephrine occasionally leads to the excretion of traces of sugar in the urine. Folin, Denis and Smillie (6) tested the urine of 70 students before and after a college examination and found glycosuria in 17 per cent of the cases immediately after the examination. Since the blood sugar must remain elevated for some time in order to lead to glycosuria and since this requires a continuous supply of epinephrine, the human adrenals are apparently capable of a prolonged acceleration of epinephrine output in response to a reflex stimulation.

Changes in basal metabolism. In table 2 are recorded 4 experiments in

which a rate of injection of 0.00005 mgm. of epinephrine per kilo per minute was followed by an increase in heat production. The basal calories represent the average of several determinations on different days including those obtained on the day of experiment. The single determination made during the 20 minutes of injection of salt solution checked very satisfactorily with the average basal value in 3 of the experiments, the difference being less than 3 per cent. In one case (March 4) the calories during the administration of salt solution were 7 per cent lower than the average basal calories. An increase in heat production was already noticeable in the metabolism period which was started 6 minutes after the beginning of the epinephrine administration. The total calories were higher in the second epinephrine period, showing that the metabolic rate continued to rise while epinephrine was being administered. On an average the total calories reached a value 13.6 per cent above the basal at the end of the injection. The return of the metabolism to normal was very rapid. In 2 cases the basal value was reached in the metabolism period starting 15 minutes after the end of the epinephrine administration; in the other two cases it took 30 minutes for the return to normal.

Two experiments with a rate of injection of 0.000025 mgm. per kilo per minute failed to show a definite rise in metabolism. In the experiment on May 13 the determination during the injection of salt solution was 9 per cent below the average basal value but the other 4 determinations, 2 during the injection of epinephrine and 2 after the injection, were all within 3 per cent of the basal, a change which cannot be regarded as significant. In the experiment on May 15 the calories in the first epinephrine period were 11 per cent above the basal, those of the second epinephrine period were however only 2 per cent higher than the basal calories. This makes it very doubtful whether the increase in metabolism in the first period should be attributed to the administration of epinephrine.

Boothby and Sandiford (7), working on trained dogs, injected epinephrine intravenously at rates varying from 0.0006 to 0.0025 mgm. per kilo per minute. The heat production rose 12 to 33 per cent during the period of injection which lasted for 6 to 13 minutes. After the injection the heat production diminished quite rapidly though the actual return to the basal level was often delayed, the metabolism remaining slightly above normal for some time. Muscular movements could be excluded as the source of the extra calorie production during the epinephrine injection. The minimal rate of injection which is just able to raise the basal metabolism of dogs was not determined by these authors. In the experiments on men very similar results were obtained with rates of injections $\frac{1}{10}$ to $\frac{1}{50}$ of those used by Boothby and Sandiford. Here too muscular movements or increased tonicity and rigidity of the muscles could be ruled out as the cause of the calorigenic effect of epinephrine.

Changes in pulse rate, respiration and blood pressure. This is shown in table 3 and requires no special discussion. With one or two exceptions an increase in pulse rate and respiration was noticeable in each experiment during the epinephrine administration. The return to normal which is not shown in table 3 required 5 to 10 minutes. The blood pressure remained uninfluenced by a rate of injection of 0.000025 mgm. per kilo per minute. A rate of injection of 0.00005 mgm. per kilo per minute caused an average rise in blood pressure of 11 and 0.0001 mgm. per kilo per minute of 15 mm. of mercury. This includes the blood pressure values obtained in 3 special experiments in which various rates of injection were tested in succession on

TABLE 3

Influence of intravenous injection of epinephrine on pulse rate, respiration and blood pressure

DATE	SUBJECT	INITIAL			SALT SOLUTION			EPINEPHRINE			AFTER EPINEPHRINE			RATE OF INJECTION
		Pulse rate	Respiration	Blood pressure	Pulse rate	Respiration	Blood pressure	Pulse rate	Respiration	Blood pressure	Pulse rate	Respiration	Blood pressure	
														mgm./kilo/minute
December 9	Sa	72	18	118	72	16	119	81	20	134	75	15	122	0.0001
November 26	Gr	78	16					85	18		75	16		0.00005
December 3	Hom	80	20					86	22		76	20		0.00005
March 4		68	15		64	14		73	16		64	14		0.00005
January 16		68	18	119	69	18	119	76	20	121	70	19	120	0.000025
December 11	Hob	72	14		72	12		78	16		72	14		0.00005
January 22		76	18	118	70	17	116	74	18	116	72	16	117	0.000025
April 22	Ka	75	15	112	77	18	111	77	19	123		18	113	0.00005
May 13		68	16		65	15		72	18		70	17		0.000025
May 15	Ma	64	15		64	16		65	18		62	17		0.000025

the same individual. In the last of these experiments which are not included in table 3 a rate of injection of 0.0002 mgm. per kilo per minute was maintained for 9 minutes which caused an elevation of the blood pressure of 23 mm. of mercury. The blood pressure generally returned to the basal level within a few minutes after the end of the epinephrine administration.

Weinberg (8) found in men that a continuous intravenous injection of 0.0005 mgm. per kilo per minute for 1 hour caused a maximal rise in blood pressure of 70 mm. of mercury; 0.0002 to 0.0004 mgm. per kilo per minute produced a rise in blood pressure of 40 to 60 mm. and 0.00005 to 0.0001 mgm. per kilo per minute of 10 to 40 mm. of mercury.

The lowest rate of injection causing a rise in blood pressure in dogs (9) was found to be 0.0002 to 0.0004, in rabbits (10, 1) 0.0008 and in rats (2) 0.001

mgm. per kilo per minute. The vascular system of man is therefore decidedly more sensitive to epinephrine than that of the common laboratory animals. This is undoubtedly the answer to the problem with which this paper started, namely, why a subcutaneous injection of epinephrine should produce a rise in blood pressure in man when it fails to do so in other species.

SUMMARY

Epinephrine was injected intravenously into normal men for a period of 30 minutes; 0.00005 mgm. per kilo per minute proved to be the lowest rate of injection which still caused a rise in blood pressure, blood lactic acid and basal metabolism; 0.000025 mgm. per kilo per minute was still able to increase pulse rate, respiration and blood sugar. After the epinephrine administration pulse rate, respiration and blood pressure returned to the original level in 5 to 10 minutes, basal metabolism in 15 to 30 minutes, while generally more than 30 minutes were required for blood sugar and blood lactic acid.

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THE FORMATION AND CIRCULATION OF LYMPH IN THE FROG

I. The Rate of Lymph Production

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Received for publication June 21, 1930

The primary circulatory purpose of the lymph vessels is still obscure. Studies on the physiology of the capillaries, carried out in recent years by Krogh, Thomas Lewis and others, have given great impetus to research on the permeability of blood vessels. The formation of lymph and its return to the blood have an important relation to permeability. Frogs have been used extensively for experiments on capillary permeability, and often results have been transferred without much consideration to the field of mammalian physiology. A thorough investigation of lymph formation and circulation in the frog promised to throw light on this somewhat dubious proceeding and also to add to our knowledge of the biology of a useful experimental animal.

Morphology. Full accounts of the anatomy of the frog's lymphatic system may be found in Gaupp (1896) and Winterstein (1925). In brief it consists of three types of structures:

1. The lymph capillaries and slightly larger vessels are found in the tissues throughout the body. The capillaries begin as blindly-ending tubes which come together forming larger vessels. In the web of the frog these vessels can be injected easily with ink. Figure 1 shows the network of lymphatics disclosed by such an injection, and figure 2, a cross sectional view of the same piece of web, shows the close relation between blood vessels and lymph vessels.

2. The larger vessels open into endothelial-lined lymph sacs or spaces which occur just under the skin, and more deeply placed among the organs. These spaces, though separated by connective tissue partitions, communicate with one another by perforations in the septa. Even the body cavity, as Schweigger-Seidel and Dogiel (1866) discovered, is physiologically part of this system of spaces.

3. Lying surrounded by the lymph of the adjoining spaces are two pairs of minute pulsating hearts. The anterior pair lie on the dorsal surface of the transverse processes of the third vertebrae, and communicate by their afferent pores with the surrounding lymph spaces. The efferent pore

opens into the anterior vertebral vein, a branch of the jugular. The posterior hearts are located more superficially, just lateral to the end of the urostyle. The pulsation of these hearts may be detected through the skin. They, too, communicate with the surrounding lymph sinuses by means of the afferent pores, and with the traverse iliac veins by single efferent pores.

The two pairs of hearts have a similar histological structure. The walls are composed of three layers, of which the inner is endothelial, the middle muscular, and the outer chiefly connective tissue. The muscular layer is arranged in long, anastomosing bundles of cross-striated fibers. From the outer layer strands of connective tissue radiate to the surrounding struc-

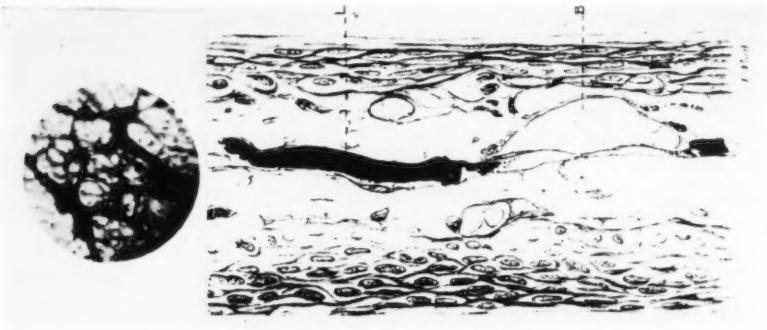

Fig. 1 Fig. 2

Fig. 1. Portion of the web of frog, with lymphatics injected. Lymphatic vessels form the black network, while blood capillaries appear as a fine gray network.

Fig. 2. Drawing of a cross section of the web shown in figure 1. *L*, lymphatic. *B*, blood vessel.

tures. When the heart contracts, these bundles, largely composed of elastic fibers, are stretched, and at diastole they return to their original length, thus causing an active dilatation of the heart. The pulsation of the hearts is not synchronous, but it is rhythmical. The usual rate is about 50 to 60 beats a minute.

From this description of the structural relationships it will be apparent that the path of lymph circulation is as follows: starting with the tissue fluid, which is made up of substances derived from the blood and of water entering through the skin, the constituents of the lymph diffuse into lymph capillaries, pass into larger lymph vessels, and from them into the lymph spaces. The lymph eventually reaches the hearts, which, with each pulsation, force it into communicating veins. It is obvious that in the frog the circulatory function of the lymphatic system is paramount. The

problem is to determine why it is necessary to have a system duplicating in so many ways the hemal system. Since the blood vessels are easily permeable to crystalloids, water and gases, and supposedly impermeable to protein, except in the liver, it is difficult to see why another route is also in use.

Material. The frogs used in the present investigation were all Rana pipiens, obtained in lots of one hundred at a time from Vermont. They were kept in tanks of cold running water until needed. At no time were signs of red-leg seen among them.

NORMAL LYMPH PRODUCTION. In Winterstein's *Handbuch* (1925) von Brücke estimates that if the stroke volume of each lymph heart is taken at as low a figure as 1 cu. mm. a beat, with 60 beats a minute the amount of fluid transported in 24 hours would be about 0.3 liter. This quantity seems tremendous in comparison with a blood volume of 2 to 3 cc., and argues for a very quick transfer of material from blood to lymph.

TABLE 1

Production of lymph

NUMBER OF FROGS			DRUG	AVERAGE WEIGHT	AVERAGE AMOUNT OF LYMPH	LYMPH PER GRAM WEIGHT	AVERAGE TIME
Male	Female	Total					
				grams	*cc.*	*cc.*	*hours*
19	14	33	Urethane	42.5	0 39	0.009	4.5
3	27	30	Curare	59.4	2.27	0.038	4.2

An experimental basis for such estimations was first offered by Isayama (1924a, 1924b). He determined the velocity of lymph production by making blood counts before and after destroying the lymph hearts. From the data obtained and from hematocrit determinations, he calculated that fluid equal in amount to fifty times the total blood plasma must pass out of the blood vessels in 24 hours. He found that within ten minutes after stoppage of the lymph hearts there was an increase in the red count of 5 to 46 per cent. After 20 minutes the blood count decreased. This he attributed to the passage of fluid back into the blood vessels, bringing about an equilibrium.

Ito (1926) repeated Isayama's work in order to find out what factors regulate this increase and decrease of cells. He found a shorter latent period of 6 to 9 minutes after destruction of the lymph hearts before the rise in the red count began. He suggested that the cauterization had a reflex effect on the capillaries, making them less permeable during the latent period. He supported this hypothesis with experimental evidence, which, according to Krogh (1929) is insufficient. An assumption involving altered permeability of the capillaries even to crystalloids is so far-

reaching that it should have a large number of experimental facts supporting it before it is accepted.

METHODS AND RESULTS. 1. *Collection of lymph.* Since the amount of fluid poured out of the vessels is obviously large, a series of experiments was planned to determine how much lymph could be collected from the subcutaneous lymph sacs in a quiescent frog. The frogs were either urethanized or curarized and were placed in a prone position tipped at an angle of about 30°, with the head up. This gravitational aid was necessary because of the pocketing of lymph by the septa between the subcutaneous sacs. Cannulas were introduced into the crural sacs at the ankles, and the lymph was collected from them. It was necessary to heparinize the frogs, to prevent clotting of the lymph. This was accomplished by giving 1 cc. of 0.1 per cent heparin solution subcutaneously about 30 minutes before starting an experiment. All the frogs were kept covered with moist gauze during the experiments, and normal saline was introduced into their stomachs so that there was no lack of liquid on which to draw. The results are shown in table 1.

It will be seen that about four times as much lymph could be collected from the curarized frogs as from the urethanized ones. This is readily explained by the stoppage of the lymph hearts when curare is used. The lymph produced cannot return through the normal channels, and since there is drainage through the cannulas, it passes off in that direction. With urethane, on the other hand, the lymph hearts are still functioning, and so, in spite of the chance for drainage, the lymph is partly returned to the circulation in the usual manner, through the lymph hearts.

Since the time in these experiments varied somewhat, they are not valuable in supplying exact data of lymph production, but they do give an idea of the amount produced in a quiescent frog during a relatively short time, and they show clearly the effect of cutting off the normal means of lymph return to the circulation.

2. *Destruction of lymph hearts.* An attempt was next made to show the effect of the destruction of the lymph hearts on the subsequent life of the frog. As the anterior hearts are very difficult of access they were not studied. It is possible to stop the lymph hearts temporarily by injecting curare, but this drug also affects the skeletal muscle. It was desirable to inject something that would not interfere with normal movements on the part of the frog. Injections directly into the lymph hearts of 95 per cent alcohol and 10 per cent formalin were tried. It was possible greatly to impair the function of the hearts with a small dose, but a dose of either alcohol or formalin increased sufficiently to cause permanent stoppage also caused death. With sub-lethal doses the hearts always partly recovered. Cauterization was next tried in five cases, under light ether anesthesia. The skin was slit with the cautery, and the point was then

plunged directly into the pulsating tissue. Several applications were needed to stop the beating. The skin was sewed up with a ribbon stitch which sloughed off with the edges of the skin in healing. When the cauterization was really successful, there was an immediate swelling around the end of the urostyle, showing that no lymph was being returned to the blood in that region. In all five cases one or both of the hearts began to beat in from one to four days after operation, when the frogs were made to exercise. The difficulty experienced in causing the complete and lasting stoppage of lymph hearts testifies to the power of the tissues both to resist destruction and to regenerate. If these experiments had been wholly successful it would be difficult to conceive of any outcome except gradual development of edema and eventual death. The length of time elapsing before death would be a measure of the efficiency of the anterior hearts to do the entire work of the body.

TABLE 2

Rate of lymph production

NUMBER OF EXPERIMENT	BODY WEIGHT	BLOOD VOLUME 4.8 PER CENT OF BODY WEIGHT	CHANGE IN 3 MINUTES	LOSS TO BLOOD IN 3 MINUTES	CALCULATED LOSS IN 24 HOURS
	grams	*cc.*	*per cent*	*cc.*	*cc.*
A 91	27.3	1.31	1.7	0.02	9.6
A 75	31.6	1.52	4.9	0.04	19.2
A 92	34.6	1.66	7.5	0.13	62.4
A 89	28.1	1.35	+0.2		

3. *Hematocrit determinations.* Another series of experiments was planned with the idea of demonstrating by a slightly different method the speed of lymph output. The principle involved was the change in hematocrit values with destruction of the lymph hearts. Frogs of 25 to 40 grams weight were anesthetized as lightly as possible with urethane, and the lymph hearts examined after anesthesia to see that they were still beating. The anterior tibial artery was cannulated and samples of blood were taken in capillary tubes inserted in the cannula. The blood was centrifugalized in these tubes and hematocrit readings were taken. The maximum quantity of blood removed for one sample never exceeded 0.04 cc.

One sample was removed before the lymph hearts were destroyed. After curarization or cauterization of the hearts other samples were taken. When curare was used an injection of 0.05 cc. of 0.5 per cent curare was made in the region of each heart. At the end of the experiment the hearts were always found to have ceased beating. When cauterization was employed only the posterior lymph hearts were cauterized, as the anterior hearts are too deeply located to be accessible without extensive trauma.

Out of a large number of experiments many must always be discarded

because of imperfections in the technique. In addition, in this case, many frogs were found to have a sluggish circulation, probably due to the season. Of those that were technically good it was found necessary to discard the curare experiments, as the injection of even 0.2 cc. of fluid made a transitory difference in the total fluid content of the body. This caused in four out of six cases an increase in plasma volume after injection, in spite of the fact that the lymph hearts were stopped. When injections of Ringer's solution were used instead of curare, as a control, the same situation obtained, showing that the addition of fluid was responsible for the increase. In two control experiments in which no injection was made,

TABLE 3

Production of edema by curarization

NUMBER OF EXPERIMENT	SEX	DRUG	WEIGHT		GAIN	
			Before immersion	6 hours after	Absolute	Per cent of body weight
			grams	*grams*	*grams*	
L 2	F.	Curare	40.0	48.6	8.6	22
L 9	F.		29.7	37.2	7.5	25
L 10	F.		23.3	28.4	5.1	22
L 3	M.		30.0	38.3	8.3	28
L 4	M.		26.7	32.9	6.2	23
L 8	M.		29.3	34.3	5.0	16
L 6	F.	Curare and urethane	64.4	70.9	6.5	10
L 11	F.		21.2	23.4	2.2	10
L 12	F.		20.6	32.0	2.4	11
L 1	M.		36.2	46.4	10.2	28
L 5	M.		28.2	37.1	8.9	32
L 7	M.		28.2	36.0	7.8	27

the plasma percentage stayed at almost exactly the same level for over an hour.

Of four cauterization experiments there was a drop in plasma volume in three cases and a rise in one case. The observed and calculated data from these experiments are given in table 2.

It may be seen that the amount of fluid lost from the blood of these frogs varies greatly, due, no doubt, to the circulatory condition of the individual frog. When the values are doubled, as would be necessary in taking into account the anterior lymph hearts as well, figures approaching those of Isayama are obtained. It seems well established that the outflow of fluid from the blood vessels and the return to the blood through the lymph hearts are rapid processes, quite unlike the familiar situation in mammals.

LYMPH PRODUCTION IN EDEMA. In order fully to appreciate normal lymph production a consideration of the pathological condition of edema is helpful.

METHODS AND RESULTS. 1. *Curarization.* The method of making a break in the chain of lymph circulation by ligating a leg was used by Moore (1915). Another type of block was used in the experiments to be reported. This was accomplished by a continued suspension of the activity of the lymph hearts with curare, which effectually prevents the return of lymph to the blood. When curarization is maintained over a long period the formation of lymph goes on as usual, and the lymph collects in the large sacs, causing edema. The frogs must be kept in water so that there is the usual absorption through the skin.

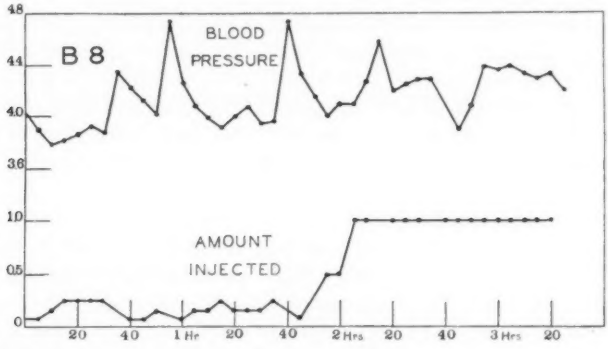

Fig. 3. Production of edema by injection of Ringer's solution. Total quantity injected was 17 cc.

Ordinate, centimeters of water.

Abscissa, time in minutes.

This method was followed with six frogs, which were weighed and given subcutaneous doses of 0.5 per cent curare. They were then placed on an inclined plane in such a way that only their heads were out of water. At the end of six hours they were taken out, dried off, and weighed. An equal number of frogs were given urethane in addition to the curare to see if the greater permeability induced by the urethane would cause a still greater gain in weight. The results are shown in table 3.

It will be seen that each frog gained a considerable amount in weight—from 10 to 32 per cent of the body weight. The addition of urethane to curare did not have the expected effect of increasing the lymph flow. The effect may have been transitory, and so not noticeable over a period of six hours, or the urethane may have had a generally depressing effect on the circulation, especially in the case of the females, whose circulation is never as vigorous as that of the males.

2. *Injection.* Another simple method of producing edema in frogs is to inject a large amount of fluid into the circulation. In a series of preliminary experiments it was found to be an easy matter to inject 15 to 20 cc. of fluid through the ventral abdominal vein during three or four hours, with only a slight displacement of blood pressure. A graph of such an experiment is shown in figure 3. The fact that the blood vessels so quickly allowed the passage of this fluid into the tissue spaces led to an attempt to find out what tissues acted as reservoirs for the quick storage of excess fluid. It was thought that the problem might be solved if a non-toxic substance could be found which could be injected with the Ringer's solution, and later precipitated in the tissues as a colored solid.

a. Prussian blue experiments. The first substance used was Prussian blue. The general procedure was to inject 3 cc. of 1 per cent solutions of potassium ferrocyanide and iron ammonium citrate in equal parts in 10 cc. of Ringer's solution. After injection one of the aortae was cannulated and the blood vessels washed out with buffered 3.5 per cent gum acacia made up in Ringer's solution, in order to prevent deposition of Prussian blue in the blood vessels themselves. The frog was then injected with hydrochloric acid, or immersed in it, or both, to precipitate Prussian blue in the tissues.

Although many variations were tried the experiment was never carried out successfully, chiefly because of the toxicity of the potassium ferrocyanide, even in very dilute solution. With the best possible results the deposit of Prussian blue, which was most evident in the skin and connective tissue, was only faint and hazy, evidently colloidal in character.

b. Bismuth sodium tartrate experiments. This substance was injected as a 2 per cent solution in Ringer's solution. The vessels were afterward washed out as in the preceding experiment, and the frogs were put into a jar of water through which hydrogen sulphide was bubbled for about an hour, in order to precipitate bismuth sulphide.

Bismuth sodium tartrate was found to be decidedly less toxic than the ferrocyanide, but the liver and spleen removed it in large amounts. After exposure to hydrogen sulphide there was usually a decided coloring of skin, connective tissue and muscles, as well as parts of the digestive tract. As it was always difficult to be sure that the blood vessels were thoroughly washed out, distribution of bismuth sulphide through the tissues was not clear cut.

One conclusion may obviously be drawn from these injection experiments: that fluid injected in quantities far greater than the blood volume of the frog did not remain long in the blood vessels. The edema fluid showed up largely in the lymph sacs, but the problem of the main places of escape was still to be solved.

c. Ringer's solution. Since the muscles make up so large a proportion

of the bulk of the body it seemed probable that they might gain in weight in edema, particularly as they are not known to possess lymphatic vessels, which would quickly drain away the fluid. Accordingly, six experiments were done in which one gastrocnemius muscle was removed from each frog to be weighed as a control. The frog was then injected through the ventral abdominal vein with 10 cc. of Ringer's solution, the injection occupying a little over an hour, and finally the other gastrocnemius muscle was removed and weighed. The frogs were prepared by curarization, to block the normal return of lymph, and novocain was used when making the incisions. The results are shown in table 4.

It will be noted that each muscle left in the frog during injection weighed more than its mate, removed before injection began. The gain is not great in proportion to the amount injected, but the fact that there is a gain in each case is significant. It is a question whether the gain in weight was

TABLE 4

Behavior of gastrocnemius muscles during experimental edema

NUMBER OF EXPERIMENT	WEIGHT OF RIGHT MUSCLE (INJECTED)	WEIGHT OF LEFT MUSCLE (UNINJECTED)	GAIN IN WEIGHT	
	grams	*grams*	*grams*	*per cent*
C 15	0.598	0.596	0.002	0.33
C 16	0.667	0.654	0.013	1.98
C 17	0.630	0.600	0.030	5.00
C 18	0.751	0.692	0.059	7.85
C 19	0.793	0.776	0.017	2.18
C 20	0.729	0.718	0.011	1.53
Average			0.022	3.15

due to the muscle tissue itself or to the connective tissue surrounding the fibers. The fact that the muscle swells only a small amount must be explained by the comparative tightness of the capillary walls.

Since the muscles were evidently not the chief storage place for water, a systematic series of weighings of injected frogs was planned. Twenty-two frogs were first injected through the ventral abdominal vein with 10 cc. of Ringer's solution, then bled and dissected, and all parts weighed. In order to evaluate the data it was necessary to have a series of normal organ weights. Although the frog is widely used for experimental purposes, little could be found in the literature on the subject of frog weights. Donaldson (1911) dealt chiefly with the central nervous system. Guthrie (1914) was concerned with the body weight as a whole. Latimer (1920) presented figures only on the viscera, and Ott (1924) was interested in the changes occurring during and after hibernation. It seemed desirable, therefore, to compile a series of weights of normal frogs from the same

group and at the same season as the injected ones. Accordingly, sixty-two frogs from the same lot were dissected and weighed along with the injected frogs.

Uniform procedure was carried out on all the frogs. Each was weighed to within 0.5 gram, given a subcutaneous injection of 1 cc. of 0.1 per cent heparin solution, and pithed. The injected frogs were pithed and dissected in the same way after the injection was finished. Particular care was taken to prevent evaporation by wrapping the frog in a moist cloth during bleeding and evisceration. The blood from the heart was removed by suction until practically no more blood came. It is impractical to try to remove all of the blood by bleeding, as dehydration of the tissues takes place in the effort to replace the lost blood. Tissue fluid and blood which escaped into the body cavity were taken up by suction as the organs were removed.

TABLE 5

Average per cent of body weight of the organs

NUMBER OF FROGS	SKIN	BONE MUSCLE N.S.	RIGHT GASTROCNEMIUS MUSCLE	HEART	LUNGS	STOMACH	INTESTINE	LIVER	SPLEEN	KIDNEYS	BLADDER	GONADS	OVIDUCTS
25 uninjected males	12.46	65.66	1.80	0.36	0.64	1.41	1.23	2.87	0.11	0.47	0.13	0.14	
10 injected males	14.40	65.34	1.82	0.34	0.66	1.43	1.12	2.66	0.09	0.43	0.16	0.15	
Difference	+1.94	−0.32	+0.02	−0.02	+0.02	+0.02	−0.11	−0.21	−0.02	−0.04	+0.03	+0.01	
37 uninjected females	9.66	47.38	1.45	0.29	0.56	1.58	1.21	2.30	0.07	0.38	0.13	19.61	5.03
12 injected females	10.79	48.84	1.44	0.29	0.57	1.57	1.33	2.04	0.06	0.36	0.17	18.48	4.92
Difference	+1.13	+1.46	−0.01	±0.0	+0.01	−0.01	+0.12	−0.26	−0.01	−0.02	+0.04	−0.13	−0.11

The various organs were transferred immediately to weighing bottles and weighed to 1 mgm. The stomach and rectum were slit open and the contents wiped out before weighing; the rest of the intestine was so nearly empty that it was not thought worth while to open it. The cloaca was included for convenience with the intestine, as was the pancreas. The fat bodies were not removed, since they were of negligible weight in the females, and in either sex represented purely inert tissue. After evisceration the skin and one or both gastrocnemius muscles were removed. The osteomuscular and nervous systems made up the carcass. To this weight were added the weights of the gastrocnemii. Table 5 shows the weights obtained and their relationship to body weights.

An examination of the table brings out the following points:

1. Influence of sex. As the weighings were done in the last two months before the normal time of emergence, the ovaries of the females were filled

with well-developed eggs, causing a considerable disproportion between the relative weights of the organs in the males and females. On the other hand the skin and osteomuscular system of the male are proportionately heavier than those of the female, and tend to balance the disproportion, so that the visceral organs are comparable in the two sexes.

2. Viscera. No significant change was seen after injection in either sex.

3. Osteomuscular system. Injection produced only an insignificant change in the gastrocnemius muscle. There is no obvious explanation of the inconsistency between the weights of the male and female carcasses.

4. Skin. There is a distinct and consistent gain in the skin of the injected frogs which is particularly significant in the light of the findings of Engels (1904) and Skelton (1927) that the areolar tissue found in the skin and elsewhere (e.g., in the septa between the lymph spaces) is a storehouse for water. It will be remembered that in the first injection experiments the colored substances were found in the loose connective tissues more than in other tissues.

5. Lymph spaces. On dissection a considerable amount of fluid was found in the lymph spaces under the skin and in the body cavity. This was always greater in the injected frogs, and undoubtedly represented the largest part of the edema fluid, though it was impossible to collect it quantitatively. This corroborates the findings of Churchill, Nakazawa and Drinker (1927) that an increased permeability to protein is characteristic of the vessels of the skin of the frog. They say: "The filtration of fluid will naturally be increased wherever the effectiveness of the already low intravascular colloidal osmotic pressure is reduced by this permeability of the capillary wall to protein."

Discussion. Even a cursory examination of the frog's lymphatic system is convincing of the fact that the mechanism is very different from that found in mammals. The chief physiological difference is the speed of lymph formation and circulation in the frog. Due to the capacious lymph spaces and the pulsating hearts, a large amount of fluid can be continuously transferred from blood to lymph and back to the blood again. This process in mammals is a very slow one. In fact it is hard to collect any lymph at all from the limbs without forced muscular activity. Obviously, in the frog there is a more primitive circulatory arrangement, with decidedly more permeable blood vessels, and an arrangement for quick removal of fluid from the tissue spaces.

The pathological condition of edema illustrates the disastrous effect of the accomplishment of one-half the lymphatic cycle, the transudation of fluid from the blood vessels, without its normal concomitant, the return of this fluid through lymphatic channels to the blood. A study of this condition is helpful in emphasizing the important place occupied by the batrachian lymphatic system. The lack of any other means of return of

the lymph, together with the large amounts involved, make it imperative that the pathway of return should be free of all obstructions.

SUMMARY

An account of the morphology of the frog's lymphatic system is given. It is shown to be adapted to the rapid circulation of large amounts of fluid.

The rate of formation of lymph was studied by means of the collection of lymph in urethanized and curarized frogs, and after the destruction of the lymph hearts by cauterization. It was found that exudation of fluid from the blood vessels normally takes place at a rapid, but variable rate, according to the circulatory condition of the frog.

A study was made of lymph production during edema induced by curarization, with consequent stoppage of the lymph hearts. Gains in weight of 10 to 32 per cent of the body weight were made by frogs over a period of six hours.

Edema was also produced by injecting large quantities of Ringer's solution into the circulation. Weights of the organs of the injected frogs were compared with weights of uninjected frogs, and the skin was found to be the only tissue absorbing fluid to any extent. Most of the fluid escaped into the lymph spaces. The inference was drawn from this fact that the skin vessels must be more permeable to fluid than other vessels.

The importance to the frog of the circulatory function of the lymphatic system is emphasized.

The problem on which this work is based was suggested by Dr. C. K. Drinker, to whom the writer wishes to express her gratitude for help and advice throughout the course of the investigation.

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THE FORMATION AND CIRCULATION OF LYMPH IN THE FROG

II. Blood Volume and Presssure

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Received for publication June 21, 1930

The factors of blood volume and blood pressure are especially important in a consideration of the formation of lymph. According to Starling's theory there is normally a balance between capillary blood pressure and the osmotic pressure of the plasma colloids. A slight excess of blood pressure causes transudation of fluid, and diminishes blood volume. It seemed worth while, therefore, to undertake the establishment of standard values for these factors in the frog.

Blood volume. All the determinations of blood volume found in the literature require the sacrifice of the animal, as they depend on total exsanguination, with volume calculated either by hemoglobin determinations or blood counts. Table 1 shows results compiled from the literature. It seemed desirable to develop a method which should not require the immediate sacrifice of the animal. At the suggestion of Dr. A. N. Richards a micromodification of the dye injection method was worked out.

Method. The preparation and use of standard color scales were described by Went and Drinker (1929). For the work with frogs, capillary tubes with an inside diameter of 0.5 to 0.8 mm. and a capacity of 0.017 to 0.042 cc. were used. The blood samples never occupied more than one-third the volume of the tubes, and the total, including a very small amount of waste, was never more than the total volume of the tubes.

Frogs were prepared by subcutaneous injections of 0.1 cc. of 1.0 per cent heparin solution, and partial immersion in a 2 per cent solution of urethane which anesthetized them in half an hour. They were kept covered with moist gauze, and the right anterior tibial artery cannulated, using the micro dissection technique described by Drinker (1927). The skin over the artery was slit with a cautery to prevent bleeding. Before introduction of the small quartz cannula, the proximal end of the artery was occluded by a silk thread, drawn over a lump of plasticine. A fine capillary tube was inserted in the cannula, into which a sample of blood was later allowed to flow.

The ventral abdominal vein was then cannulated by the same technique. The dye-filled cannula was furnished with a short piece of clamped rubber tubing, to allow the insertion of a syringe needle. When the cannula was satisfactorily adjusted, the vein, occluded by a single twist with a silk thread, was released, and 0.1 cc. of 0.1 per cent vital red HR was injected through the cannula from a tuberculin syringe. After a mixing time of two minutes, a sample of blood was taken in the capillary tube from the anterior tibial artery. The tube was sealed and mounted in a perforated cork which fitted into a metal centrifuge cup. After centrifugalization of the blood, the color of the plasma was compared with the color scale, arranged in units of milligrams per cent of vital red in different dilutions of horse serum. From the color comparison and the proportion of plasma

TABLE 1

Blood volume

OBSERVER	BLOOD WEIGHT AS PER CENT OF BODY WEIGHT	METHOD
Fry, 1914	4.64	Hemoglobin
Ranke, 1871	6.52	
Welcker, 1858	5.50	
Gaule, 1889	4.20	Blood counts
Gürber, 1889	3.24	

to whole blood, the calculation of blood volume was made. A sample protocol follows:

Experiment A 100. Male. Weight 26.2 grams. May 19, 1930.

11:00 Injected 0.1 cc. of 1 per cent heparin. Put frog into solution of 2 per cent urethane.

11:35 Anesthetization complete. Cannulated anterior tibial artery and ventral abdominal vein.

11:58 Injected 0.1 cc. of 0.1 per cent vital red HR into the ventral abdominal vein.

12:00 Sample of blood taken.

Color of plasma = 7 mgm. per cent standard
Total length of column = 20.0 mm.
Column of plasma = 13.0 mm. = 65 per cent
Column of cells = 7.0 mm. = 35 per cent

$$\frac{100 \times 0.1}{7} = 1.43 \text{ cc. Volume of plasma only}$$

$$\frac{100 \times 1.43}{65} = 2.2 \text{ cc. Total blood volume}$$

Results. Six successful determinations of blood volume were made. The data are given in table 2.

These results are consistently higher than those obtained by other methods. Since this method has been found by Went and Drinker to be reliable with other animals, and since it is theoretically sound, the results are offered as being worthy of consideration. The method has the advantage of measuring blood volume in the living animal, as none of the other methods do, and it makes possible a repetition of the determination after an experimental period.

TABLE 2

Blood volume by the dye injection method

NUMBER OF EXPERIMENT	WEIGHT	COLOR	PLASMA	BLOOD VOLUME		
	grams	*mgm. per cent*	*per cent*	*cc.*	*grams*	*per cent of body weight*
A 100 M	26.2	7	65.0	2.20	2.31	8.81
A 101 F*	25.0	6	67.2	2.47	2.59	10.36
A 103 M	28.2	6	76.4	2.18	2.29	8.12
A 105 F*	27.3	6	66.0	2.53	2.66	9.74
A 106 M	30.0	7	68.9	2.07	2.17	7.23
A 108 M	26.0	7	70.2	2.03	2.13	8.19
Average...	27.1			2.25	2.36	8.74

* Ova had been expelled.

TABLE 3

Blood pressure

INVESTIGATOR	NUMBER OF MEASUREMENTS	BLOOD VESSEL	SYSTOLIC BLOOD PRESSURE*	
			mm. Hg	*cm. H_2O*
Jacobj (1900)		Left aorta	40	52
Jacobj and Römer (1911)		Left aorta	30–35	(40–47)
Kuno (1914)	12	Pulmocut. artery	20–46	(27–62)
Landis (1926)	22	Mesent. artery	(18–37)	24–50
Hayman (1927)	39	Abdominal aorta	(16–45)	21–61
Bieter and Scott (1928)		Leg cuff	(19–26)	25–35

* The figures in parentheses are calculated.

BLOOD PRESSURE. Blood pressure determinations have been made by a number of observers, generally to throw light on the problem of permeability, excretion or the action of drugs. The data accumulated are valuable as illustrating the fact that there is no set level for blood pressure in the frog. It can and does vary within wide limits, in different individuals, without seeming to cause any untoward effects. Table 3 summarizes figures on arterial pressure taken from the literature.

The present study was carried out first, to determine the normal arterial

blood pressure of frogs; second, to see whether, under anesthesia, a level could be maintained for several hours; and third, to demonstrate the effect of exercise on blood pressure.

Method. The direct method was used throughout, and the data were obtained from the anterior tibial artery. The use of 5 per cent barbital in doses of 1 cc., introduced subcutaneously, was adopted in sustained experiments. Urethane and curare are not suitable as immobilizing drugs in such experiments, since the former causes vasodilatation and the latter stops the action of the lymph hearts, and so prevents the return of lymph to the circulation. An injection of 1 cc. of 0.1 per cent heparin was given half an hour before starting the experiment

The anterior tibial artery of the right leg was cannulated by the technique described under blood volume, and the cannula was connected with a water manometer filled with heparinized Ringer's solution, made up

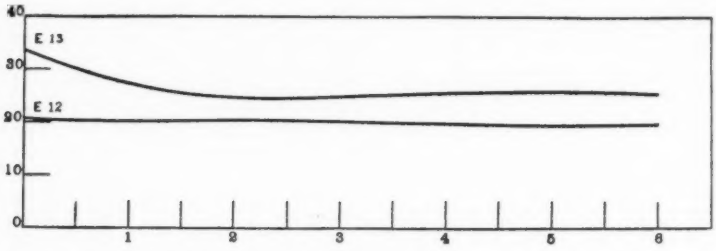

Fig. 1. Systolic blood pressure in the anterior tibial artery, taken every minute for six hours.

Ordinate, centimeters of water. Abscissa, time in hours.

according to Clark's (1913) formula. A syringe, also filled with the solution, was connected with the manometer and cannula by means of a T-tube. This syringe, mounted with another, according to the description of Richards, Barnwell and Bradley (1927), was furnished with a screw drive for the plunger, making possible very fine adjustments of pressure.

When the cannula was inserted in the artery the pressure in the manometer was run up to about 25 to 30 cm. by means of the syringe. The thread occluding the artery was then released, and an adjustment of the pressure was made so that a maximal pulsation was visible through the microscope in the neck of the cannula. At this point the systolic pressure was read on the manometer. Corrections were made for capillarity and hydrostatic pressure. In a number of cases, two of which are shown in figure 1, the pressure was read and recorded every minute for six hours.

It was necessary to observe care not to introduce small quantities of Ringer's solution in making the adjustment with the syringe for each read-

ing. A record was kept of the position of the plunger at the beginning and end of each observation. In the two cases shown in figure 1 the syringe showed 0.4 and 0.5 cc. respectively to have been injected, but in each instance this was considerably less than the loss by evaporation from the skin, as shown by the weights. This loss occurred, even though the frog was completely covered with moist gauze.

The effect of exercise on blood pressure was demonstrated by a series of experiments in which one leg was stimulated at intervals with a tetanizing current sufficient to cause marked contraction of the extensor muscles. The frog was prepared as usual for blood pressure determinations. The right anterior tibial artery was cannulated and attached to a water manometer, and by a T-tube, to the regulatory syringe. The left leg was tetanized while the blood pressure was watched.

TABLE 4

Initial blood pressure values

NUMBER OF EXPERIMENT	PRESSURE	DRUG
	cm. H_2O	
1 F	29.0	Curare
9 M	39.2	
10 M	39.7	
4 M	42.0	
3 F	16.1	Urethane
2 F	32.0	
11 M	14.8	Barbital
12 M	19.6	
13 F	32.6	

Results. A series of initial values for blood pressure is given in table 4. These particular figures were selected from a large number because they were considered technically good, and because the frogs were all unusually vigorous and active.

A variation in initial values from 14.8 to 42 cm. of water was seen. This corroborates the data obtained on other arteries (see table 3), and confirms the statement that blood pressure in the frog is extremely variable between individuals.

In the cases in which blood pressure was read and recorded every minute for long periods of time, the pressure varied almost not at all in the quiescent frog. A graphic record of two such cases is shown in figure 1.

The factor of exercise is of especial importance in the frog, a sluggish animal, capable at times of sudden and violent exertion, as in leaping and swimming. The rise in blood pressure during exertion is translated in the

capillary bed into increased filtration, so that more lymph is produced, and blood volume decreases. This reciprocal relationship is dramatically illustrated in figure 2, a graphic record of an experiment showing that tetanization produces a corresponding rise in blood pressure, which is, however, followed immediately by adjustment to a lower level, indicating increased transudation of fluid.

Discussion. The relation of blood volume and pressure to the formation and circulation of lymph is obvious. Alterations in blood pressure upset the balance between the osmotic pressure of the plasma proteins and the capillary blood pressure, and cause rapid lymph formation, with temporary lowering of blood volume. Owing to the exceptionally rapid system

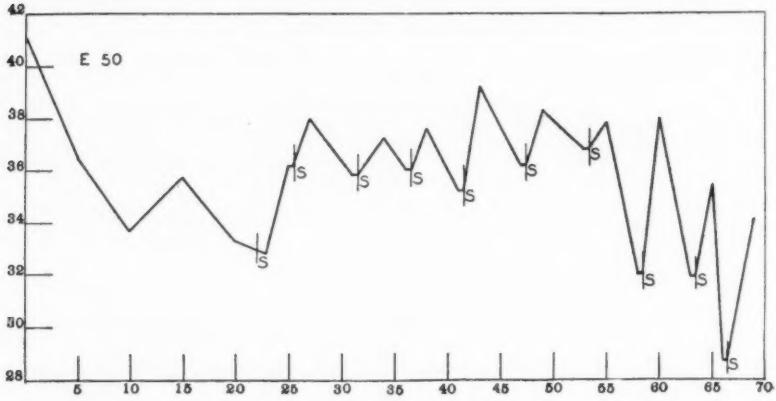

Fig. 2. The effect of exercise on arterial blood pressure. *S* indicates stimulation of one leg with an induced current. Blood pressure is taken in the anterior tibial artery of the opposite leg.

Ordinate, centimeters of water. Abscissa, time in minutes.

of lymph return in the frog, described in an earlier paper (Conklin, 1930), the lymph quickly re-enters the blood stream and makes up the loss in volume. A high degree of permeability of the blood vessels imperatively demands a speedy return of the fluid lost to the blood. The difference is marked between this sort of system and the laboriously slow return of lymph from the periphery in mammals. As a means of rapid drainage the lymphatic system of the frog is far more effective. What the frog lacks in ability to hold fluid in the blood vessels, it makes up in speed of return of the transudate through the lymphatic system. If this system fails, the life of the frog is immediately and seriously jeopardized. It will eventually die because of the segregation in its own lymph spaces of valuable constituents of the blood.

SUMMARY

1. A new method is described for determining blood volume in the frog. This method is a micromodification of the dye injection method, and does not require the immediate sacrifice of the animal.

2. The values obtained by this method are higher than those reported by earlier observers. The average blood volume is 8.74 per cent of the body weight.

3. A method is described for the direct observation of blood pressure in the anterior tibial artery of the frog.

4. Initial values for blood pressure varied in nine cases from 14.8 to 42.0 cm. of water. When blood pressure is watched over a period of several hours it is maintained in quiescent frogs at practically the initial level.

5. Exercise causes an immediate rise in blood pressure, with a rapid readjustment to the former level, due to transudation of fluid.

6. Blood volume and pressure are mutually dependent and intimately related to the formation of lymph. Increase in blood pressure brought on by activity concentrates the blood, forcing more fluid out of the capillaries, and so increasing the flow of lymph. Quick return of lymph to the blood is provided for by the lymph hearts.

The writer wishes to express her thanks to Dr. C. K. Drinker for his interest and helpful suggestions in carrying out this work.

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THE FORMATION AND CIRCULATION OF LYMPH IN THE FROG

III. The Permeability of the Capillaries to Protein

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Received for publication June 21, 1930

It has been shown that the frog normally produces lymph in very large quantities, and that this lymph is quickly returned to the blood by the lymph hearts. It has also been shown that the skin vessels are highly permeable to water. Even when the blood pressure is at the level maintained during quiescence, there is a copious production of lymph, and increase of pressure, as during exercise, causes a still greater exudation from the blood vessels (Conklin, 1930a, b). The accepted theory of lymph production (Starling, 1896) assumes a balance between colloid osmotic pressure of the blood and capillary blood pressure, with the impermeability of the capillaries to protein as a necessary corollary. Since the capillaries of the skin cannot reasonably be expected to have a higher blood pressure than those of the viscera or muscles, the question arises whether the colloid osmotic pressure is sufficiently high to maintain the required balance. If protein does pass through the capillaries it immediately lowers the effective osmotic pressure of the blood, and increases the osmotic pressure of the tissue fluid, thus drawing water out from the blood. Experimental work undertaken with this question in mind has resulted in the conclusion that the skin capillaries of the frog are permeable to protein, and that the blood is continually losing this substance, only to have it returned through the lymphatic system.

In the first edition of *The Anatomy and Physiology of Capillaries*, Krogh (1922) stressed the dependence of increased permeability on mechanical stretching of the capillaries. This was based on experiments in which stasis, a packing of the corpuscles with filtering off of fluid, was brought about by various stimuli causing dilatation. Krogh and Harrop (1921) had previously stated that capillary pressure had very little effect on permeability, since urethane, which leaves the arterioles contracted, produced stasis as easily as did nerve stimuli which dilated arterioles and capillaries. By the time of publication of the second edition of Krogh's book (1929) he had somewhat modified this thesis, largely on account of Landis' work, though he still found a striking coincidence between increase in diameter and increase in permeability.

Landis' (1926) chief contribution to the subject consisted in the direct measurement of blood pressure in the mesenteric capillaries. By means of the Chambers micromanipulator he was able to measure pressures in individual capillaries, arteries and veins with great accuracy. He found the capillary pressure to vary in the majority of cases between 10 and 14.5 cm. of water. This was in striking agreement with the osmotic pressure of the plasma colloids as reported by White (1924), who found an average value in three cases of 11 cm. of water. Landis also found that the pressure might not only differ in the different capillary branches of the same arteriole, but that it might also differ in the same capillary at short time intervals. As he says: "Pressure may be above or below the reported pressure of the plasma colloids throughout the whole length of any capillary in successive moments."

Landis (1927a) found in experiments on dilatation versus heightened pressure that rise in capillary pressure is accompanied by a slow concentration of corpuscles, but that the much more marked concentration and stasis produced by urethane could be brought about without an increase in diameter of the capillaries. He concluded that urethane in the strengths used by Krogh (5 to 25 per cent) is poisonous to the endothelial wall, and causes greater permeability for this reason rather than because of dilatation. Further experiments with injection of dye (Landis, 1927b) showed that the rate of diffusion of a dye solution through the capillary wall depended on capillary pressure rather than diameter. At 11.5 cm. of water, fluid moved neither in nor out of the capillaries. When pressure was increased above this value there was filtration; when it was decreased there was absorption. Poisoning of the endothelium by narcotics or other drugs, or lack of oxygen (Landis, 1928) produced large increases in permeability.

Landis' results are significant in confirming the Starling hypothesis at least for the capillaries of the frog's mesentery, but it must not be forgotten that capillaries may differ markedly in permeability. For instance, it has been known since 1894, when Starling investigated the formation of lymph, that the capillaries of the liver and intestinal mucosa of mammals are permeable to protein. A rise in blood pressure in the liver causes a great outpouring of lymph, which is practically as highly proteinized as the blood itself. It is quite possible that capillaries may show a differential permeability to protein.

The passage of protein through the skin capillaries of the frog was brought out in 1927 by Churchill, Nakazawa and Drinker. They found lymph in the subcutaneous spaces to contain from 0.29 to 2.17 per cent of protein, and to have an osmotic pressure of 42 mm. of water. They also made nine determinations of the osmotic pressure of the blood, and obtained an average value of 71 mm. of water, somewhat lower than White's

figure. This would mean an effective osmotic pressure of the plasma proteins of only 29 mm. of water. Churchill and his collaborators compare this with the average capillary pressure of 122 mm. found by Landis, and conclude that this explains the large volume of lymph produced. The effect of protein in concentrations up to 2.17 per cent outside the blood vessels increases the filtration of fluid which is already large, due to excess of blood pressure over the colloid osmotic pressure of the blood.

The size of particles which will pass through the capillary endothelium has not been well defined. Krogh (1929) found that starch was held back by normal capillaries, but will pass through when they are strongly dilated. Since soluble starch particles are about 5μ in diameter, some conception is possible of the size of particles which can pass through normal capillaries.

It is well known that colloidal substances injected subcutaneously are absorbed very slowly. This was proved by Schulemann (1917) for colloidal dyes, which remained at the site of injection for long periods. The familiar slow absorption of serum as in antitoxin injections was illustrated experimentally by J. H. Lewis (1921), who identified injected serum by immunological methods. The specific protein was very slowly absorbed, but was recognized in less than an hour in the lymph, while it took three and a half hours to appear in recognizable quantities in the blood. The problem of the passage of colloids from the tissues into the blood stream must be recognized as quite distinct from that of the exit of colloids from the blood stream into the tissue spaces.

THE PASSAGE OF COLLOIDS FROM BLOOD TO LYMPH. The experiments planned to test the passage of protein out of the blood and into the lymph were divided into two groups, the first dealing with the washing out of the normal plasma proteins, and the second concerned with injected proteins.

Methods and results. 1. *Blood washing experiments.* In this group the plasma proteins were washed out of the blood by the injection into the circulation of large quantities of Ringer's solution. The frog was prepared with subcutaneous injections of heparin and curare. In some cases the forebrain was pithed, and in others novocain was used at the line of incision. The ventral abdominal vein was cannulated for injection of Ringer's solution, and the anterior tibial artery was also cannulated and connected with a water manometer, so that blood pressure readings could be taken. Cannulas were inserted into the crural sacs at the ankles, and the whole preparation was tipped, so that gravity would aid in the collection of lymph. Ringer's solution was injected into the blood in very small amounts at intervals by means of a mechanical injector, and samples of lymph were taken off and analyzed refractometrically for protein.

Ten successful experiments were carried out, six of which are shown in figure 1. A typical protocol follows:

Experiment J2. March 15, 1929. Female; weight, 55 grams.

9:30. 1 cc. of 0.1 per cent heparin and 0.2 cc. of 0.5 per cent curare injected subcutaneously. Cannulated ventral abdominal vein and anterior tibial artery. Inserted four cannulas under the skin, at ankles and back. Tilted frog, head up.

INJECTION OF RINGER'S SOLUTION	TIME	BLOOD PRESSURE	AMOUNT OF LYMPH	PROTEIN OF LYMPH
cc. per 5 minutes		*cm. H_2O*	*cc.*	*per cent*
	10:45	19.1	0.1	0.66
	10:48	21.6		
0.25	10:51	21.2		
	10:54	25.1		
	11:00	26.8		
	11:03	25.6		
	11:06	25.3		
	11:09	34.7		
	11:12	32.7		
0.5	11:15	33.2	0.1	2.47
	11:18	33.0		
	11:21	33.0		
	11:24	32.7		
	11:27	31.9		
	11:30	33.0	0.2	2.27
0.75	11:33	31.5		
	11:36	31.4		
	11:39	31.1		
	11:42	32.8		
0.25	11:45	32.9	0.55	2.03
	11:50	32.1		
	11:53	32.0		
	11:56	33.3		
	11:59	32.2		
	*12:00		0.8	1.65
	12:02	34.4		
	12:05	33.4		
	12:08	33.0		
	12:11	32.5		
0.75	12:14	33.1		
	*12:15		1.0	1.32
	12:17	30.8		
	12:20	32.2		
	12:23	31.5		
	12:26	31.9		
	12:29	34.7		
	*12:30		1.2	1.05
	12:36	30.6		

* No injection.

Concluded

INJECTION OF RINGER'S SOLUTION	TIME	BLOOD PRESSURE	AMOUNT OF LYMPH	PROTEIN OF LYMPH
cc. per 3 minutes		*cm. H_2O*	*cc.*	*per cent*
	12:39	31.0		
	12:42	32.3		
	12:45	33.2	1.5	0.83
	12:48	31.1		
	12:51	35.4		
	12:54	34.0		
0.75	12:57	33.6		
	1:00	34.0	1.0	0.81
	1:03	29.7		
	1:06	30.2		
	1:09	32.1		
	1:12	31.1		
	*1:15		1.6	0.66

Total injection, 30.0 cc. At end of experiment blood would not run from artery. Sample taken from heart. Plasma proteins found to be 0.18 per cent.

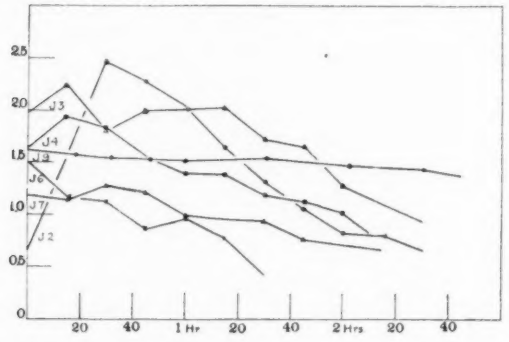

Fig. 1. "Blood-washing" experiments. The curves represent lymph protein, determined during injection of Ringer's solution into the blood.
Ordinate, per cent of protein. Abscissa, time in minutes.

This protocol illustrates several points: In the first place, in spite of an injection of 30 cc. of fluid, the blood pressure was maintained at a fairly even level, after the initial rise. The only explanation of this fact is that fluid poured out of the blood vessels almost as fast as it was injected. In the second place, the lymph which was collected showed a steadily decreasing content of protein, after the marked rise in the second sample. Two and a half hours after the first injection the lymph protein had dropped to

0.66 per cent, the original value. In the third place, the plasma proteins had gone down almost to the disappearing point—0.18 per cent. This is far below any figure given for normal frogs. In another series of nine frogs the plasma protein was 2.23 per cent. The figures given by White (1924) and Churchill, Nakazawa and Drinker (1927) are of the same order. There is no doubt that the protein can be almost completely washed out of frog's blood by the injection of Ringer's solution.

A single experiment was done in which the frog was allowed to recover, after reducing the lymph protein to 0.66 per cent. The next day a sample of blood was taken from the leg. The plasma proved to have 1.08 per cent of protein, indicating that the blood had not yet been able to make up the deficiency.

2. *Injection experiments.* This group of experiments was designed to show what proteins will pass easily through the capillary wall. In some cases, where the substance could be identified by its color, a solution was injected into the blood and samples of lymph were taken off at intervals and examined for traces of color. In the case of colorless proteins the procedure was to wash the plasma proteins out of the blood with Ringer's solution, then inject the test solution and determine protein in the lymph refractometrically. An increase of protein in the lymph could only mean that it was coming from the blood. This last method has the disadvantage, that after extensive flushing with Ringer's solution the capillaries are undoubtedly more permeable than in an untreated frog. Nevertheless it is instructive to know what size molecules will pass through even under those circumstances. The same technique was employed as in the blood-washing experiments, except that blood pressure was not recorded.

Plasma proteins. The fact that it was necessary to heparinize frogs to prevent the coagulation of lymph proves the presence of fibrinogen in normal lymph. This is true of frogs which have not been subjected to any operative procedure. The presence of albumins and globulins in normal lymph was demonstrated by two experiments in which lymph was collected from intact frogs, whose stomachs were kept filled with Ringer's solution to provide p' nty of fluid. These proteins could be salted out with ammonium sulphate from the samples collected after 45 minutes. The lymph, therefore, contains all of the normal plasma proteins.

Egg albumin. The blood-washing process was carried out in three experiments on egg albumin. After drastic reduction of the circulating proteins, 2 per cent buffered egg albumin solution, with a refractometric index equivalent to 1.65 per cent protein, was injected in small amounts. In one case there was no rise in the lymph protein during 15 minutes of albumin injection. In the second, there was a very slight rise in the same length of time. In the third, number M 17, there was a marked rise in 40 minutes. The percentage continued to rise during the duration of the

experiment, indicating passage of protein from the blood. It is evident, however, that egg albumin molecules do not pass through capillaries with ease.

Gelatin. The same method was followed in two experiments with gelatin. A 1 per cent buffered solution of the gelatin, with a refractometric index equivalent to 1.63 per cent protein was injected after reducing the lymph protein to 0.66 per cent in one case and 0.76 per cent in the other. In the first case the lymph protein did not rise above 0.66 per cent, though the injection was continued for 1 hour and 33 minutes. In the second case, there was a rise to 0.96 per cent in 25 minutes, with a slight fall at the end of the experiment. As in the case of albumin, gelatin evidently passes through capillaries with difficulty, and only when they have been made abnormally permeable.

Hemoglobin. One experiment was done with injection of water only, with the idea of hemolyzing the frog's own blood, and watching for the passage of hemoglobin into the lymph. In an hour and a half the lymph had a distinct red tinge. Microscopic examination revealed no red cells, so that the color must have been due to hemoglobin from the laked blood. In two other experiments a solution of hemoglobin was prepared from human blood and made up to 0.65 per cent sodium chloride before injecting. Colorless lymph was collected as a control before injecting. A distinct red tinge could be seen in the lymph in 20 minutes, and in one hour the lymph was a bright red, indicating the easy passage of hemoglobin through the capillaries. No doubt small amounts passed through almost immediately, but it took some time to impart a discernible tinge to the lymph as a whole.

Hemocyanin. A sample of purified Limulus hemocyanin, "about 10 per cent," was obtained from Dr. A. C. Redfield. This was injected and recognized in the lymph after 30 minutes. Since the color was not a pronounced blue it is quite probable that it had come through at a much earlier time, but in quantities too small to recognize. Busycon purified hemocyanin, obtained from the same source, appeared in the lymph after 32 minutes. Busycon serum, containing the native hemocyanin, was diluted to 0.7 per cent salt concentration, and injected. The molecule is supposedly many times larger than the purified hemocyanin, due to aggregation, but this passed into the lymph in 30 minutes. It seemed highly probable that this invertebrate serum contained lymphagogues of the first class which poisoned the capillary wall and so rendered it permeable to the larger molecules. This was proved by later experiments to be the case, when injection of this serum almost immediately rendered the capillaries permeable to starch molecules.

Colloidal carbohydrates. Three experiments were done to test the permeability of the capillaries to dextrin and starch. A 1 per cent buffered

dextrin solution was injected in amounts totalling 17 cc. over a period of 1 hour and 18 minutes. At intervals the lymph was tested with iodine for dextrin, but at no time did it give a positive test. In another experiment, 1 per cent buffered starch solution was injected in amounts totalling 26 cc. over a period of 1 hour and 42 minutes, before starch finally appeared in the lymph, as indicated by the iodine test. When, however, the capillaries were first made more permeable by injections of Ringer's solution amounting to 20 cc. in the course of 52 minutes, the injection of starch following this treatment resulted in a positive test in the lymph after 22 minutes.

Purified 3 per cent gum acacia, buffered and made up to 0.7 per cent sodium chloride, was also injected. The presence of acacia in the lymph

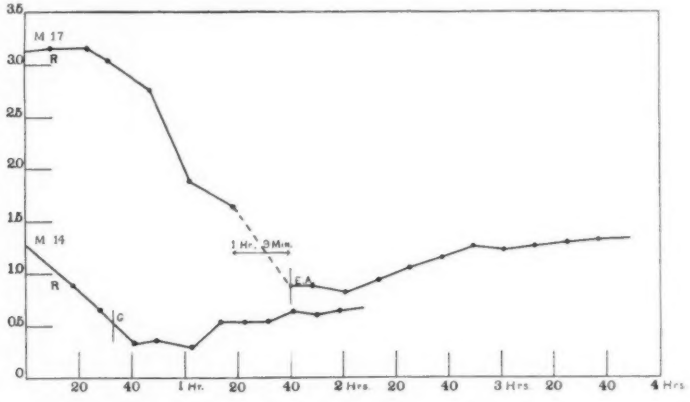

Fig. 2. The passage of protein through capillaries. The curves represent lymph protein.

Ordinate, per cent of protein. Abscissa, time in minutes. *R*, injection of Ringer's solution. *E. A.*, injection of egg albumin solution. *G*, injection of gelatin solution.

was determined by the method of Corper and Rensch (1921). When acacia only was injected in small amounts it did not appear in the lymph. When, however, 10 cc. of Ringer's solution were injected, followed by the acacia solution, very small amounts of the gum could be detected in the lymph in 10 minutes. Collection of lymph after cessation of injection resulted in acacia-free lymph. Here, again, the capillaries must have been made more permeable by the injection of Ringer's solution. Figures 2 and 3 summarize the results of this series of experiments.

THE PASSAGE OF COLLOIDS FROM LYMPH TO BLOOD. A series of experiments was planned to discover whether frog capillaries, obviously permeable to protein in one direction, could absorb colloidal molecules from the tissue fluid. This could be ascertained by the injection of a dye or a

colored protein into the lymph sacs of a curarized frog, watching for its appearance in the lymph.

Method. Frogs were curarized and heparinized, and the anterior tibial artery of the right leg was cannulated, using novocain at the line of incision.

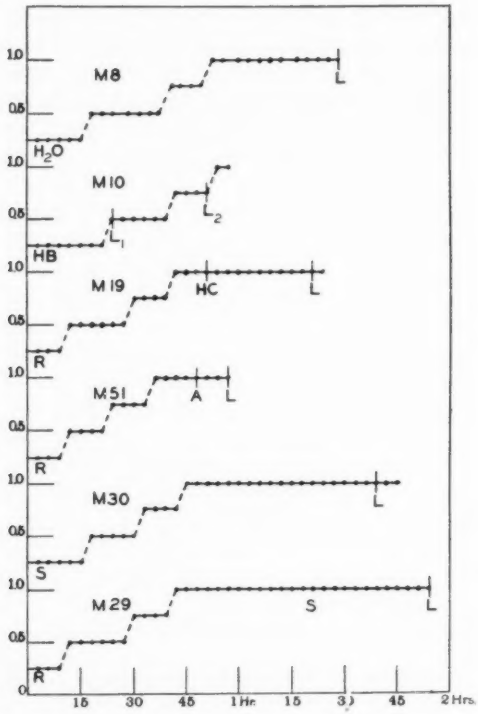

Fig. 3. The passage of colloids through capillaries. Curves represent injections into the ventral abdominal vein. Ordinates, amounts injected in cubic centimeters. Abscissa, time in minutes. Injected substances: H_2O, injection of water, with hemolysis, and appearance of hemoglobin in the lymph. *HB*, hemoglobin. *HC*, hemocyanin. *R*, Ringer's solution. *A*, gum acacia solution. *S*, starch solution. *L*, appearance of injected substances in the lymph.

One cubic centimeter of vital red or frog hemoglobin solution was then injected into the lymph sac of the left ankle. Blood samples were taken in capillary tubes from the artery at 5 or 10 minute intervals. The blood was centrifugalized, and the plasma examined against a white background for traces of color.

As checks on these experiments a number were done where urethane was

used in place of curare. This would not interfere with the normal return of dye to the blood through the lymph hearts. Two experiments were done with the muscles and skin of the left thigh tied off and divided, leaving only the femur, the femoral artery and vein and the sciatic nerve connected with

TABLE 1

Passage of colloids from lymph to blood

DRUG	NUMBER OF EXPERIMENT	DURATION OF EXPERIMENT		COLOR IN PLASMA	LYMPH HEARTS AT END OF EXPERIMENT
		hours	*minutes*		
I. Vital red HR					
Curare	K 7 M	1	34	None	Not beating
	K 13 M	1	15	None	Not beating
	K 22 F	1		Faint, in 15 minutes	Beating slightly
	K 52 M	1	13	None	Not beating
Urethane	K 17 M	4		Faint, in 10 minutes	Beating, urine colored
	K 18 M	3		Marked, in 10 minutes	Beating strongly
	K 21 F		45	Marked, in 9 minutes	Beating
Barbital (operated)	K 15 M	1	38	None	Beating, no color in urine
	K 24 M	1	30	None	Beating. Capillary circulation in web good
II. Hemoglobin					
Curare	K 29 M	1	25	None	Not beating
	K 30 M		55	Faint, in 25 minutes	Beating irregularly
	K 31 M	1	30	None	Not beating
	K 50 M	1	15	None	Not beating
	K 51 F	1	10	None	Not beating
Urethane	K 32 M		30	Marked, in 20 minutes	Beating

the leg. The dye was then injected into the leg as usual, and blood samples were taken from the other leg. In the second one of these experiments, K 24, the circulation in the web of the left foot was watched under the microscope at intervals, beginning five minutes after the operation was over, and it was seen at all times to be in excellent condition, with blood flowing at the usual rate and no stasis visible. In each case samples of blood were withdrawn at intervals for one and one-half hours.

In three experiments the dye or hemoglobin solution was forced down into the web, where the close relation between the lymphatic and blood capillary nets could be seen microscopically. In these cases particularly there seemed to be no physical barrier to the passage of dye except the endothelial walls of the two capillaries.

Results. The results of these experiments are embodied in table 1.

Discussion. In the blood-washing experiments it is seen that when the return of lymph to the blood is shut off by curarizing the lymph hearts, and the normal rate of filtration from the blood vessels is increased by Ringer's solution, the blood can be practically deproteinized in a very short time. It is also obvious that enormous amounts of fluid can pass through the capillaries in a very short time. It is usually necessary to increase the unit amount as the experiment proceeds, or the increased exudation results in a marked lowering of the blood pressure. Thus, the injection starts out with a unit amount of 0.25 cc., which is increased to 0.5 cc., 0.75 cc., and finally 1 cc., with the same intervals, and without much increase in blood pressure.

TABLE 2

Molecular weights of the proteins

PROTEIN	PROBABLE MOLECULAR WEIGHT	INVESTIGATOR
Egg albumin	34,500	Svedberg and Nichols (1926)
Serum albumin	67,500	Svedberg and Sjögren (1928)
Hemoglobin, horse	66,800	Svedberg and Fähreus (1926)
Hemocyanin, Limulus	90,800	Cohn, Hendry and Prentiss (1925)
Serum globulin	103,800	Svedberg and Sjögren (1928)
Gelatin	150,125	Cohn and Conant (1926)
Hemocyanin, Limulus	2,040,000	Svedberg and Heyroth (1929)

The molecular weights of the proteins used in the injection experiments are given in table 2. All of these substances were found to pass through the capillaries, some of them with difficulty. The part played by the large injections of fluid in increasing permeability is not definitely determined. It is quite probable that the capillaries do respond to such treatment by increased permeability. The experiments with colloidal carbohydrates, which will pass through only after extensive flushing with Ringer's solution, would indicate that the same may be true of protein solutions. It is difficult to draw the line between normal and abnormal permeability. The fact that the plasma proteins, fibrinogen, albumins and globulins are found normally in the lymph indicates easy passage of these molecules from blood to tissue fluid. The comparative ease with which hemoglobin and hemocyanin pass into the lymph indicates that they are at least near the limit for normal permeability.

In connection with the statement of Drinker (1927) that the capillaries of the frog's foot "leak protein-containing fluid at all diameters," Landis (1928) suggests that the tissue fluid immediately surrounding the capillaries may differ widely in composition from the lymph even of the same area, and that the original filtrate might be modified considerably in its passage through the tissue spaces, the smaller lymph capillaries and the lymph glands. When the very close anatomical relationship between blood and lymph capillaries in the web and skin of the leg is taken into account, it seems doubtful that any great difference can exist between tissue fluid and lymph there. The only agency present which could change the composition of the tissue fluid, aside from the metabolic changes taking place in the tissue itself, is the endothelial wall of the lymph capillary. The properties of this tissue as an active or passive barrier are as yet unknown. As the frog possesses no lymph glands, there can be no question of modification of the lymph by that means.

If the data contained in table 1 are examined it will be seen that when the lymph hearts were definitely stopped with curare, no color appeared in the lymph. When there was a partial recovery from curare color did appear in the lymph, but only after a considerable interval, as compared with its appearance after urethane. In the experiments numbered K 15 and K 24, where there was no chance for return of lymph except by absorption into the blood vessels, there was no sign of color for an hour and a half.

It is probable that the passage of protein in one direction only can be explained by pressure and osmotic relations. In these experiments water was continually passing out of the blood vessels, so that the hydrostatic pressure gradient was always towards the tissues, rather than away from them. Also, the blood was higher in protein than the lymph, so that diffusion of this substance would not naturally take place from lymph to blood. Experiments are planned for the future in which a reversal of the normal condition will be brought about, by depleting the blood protein in a curarized frog and then placing in the lymph sacs large quantities of frog plasma. If, in such an experiment, the blood does not take up protein, there will be grounds for postulating a valve-like permeability of the capillaries. Whatever the mechanism may be, the fact remains that under usual circumstances protein derived from the blood must return by the lymphatic route. When this route is blocked, as by stopping the lymph hearts, protein accumulates in the lymph spaces, and is temporarily lost to the bodily economy. This is conclusive evidence of the importance to the frog of its lymphatic system.

SUMMARY

Capillaries of the frog's skin are normally permeable to protein, but this substance, once it has left the capillaries, does not return through their walls, but takes the lymphatic route, passing through the lymph sacs, and

eventually being returned to the blood by the lymph hearts. Experimental proof is offered as follows:

1. Lymph normally contains fibrinogen, albumins and globulins, the proteins of the plasma.

2. The plasma proteins may be almost entirely washed out of the blood by injecting large amounts of Ringer's solution into the circulation of curarized frogs. The lymph hearts are stopped by the curare, and the lymph is drained off through cannulas inserted under the skin. The percentage of protein in the lymph rises quickly, then falls slowly as the blood is depleted. At the end of the experiment both blood plasma and lymph are very low in protein.

3. Foreign proteins injected into the circulation pass through the skin capillaries and may be recovered in the lymph. Hemoglobin and hemocyanin pass through with relative ease, while egg albumin and gelatin will only pass through after the capillaries have been rendered abnormally permeable with the injection of Ringer's solution. The latter is also true of colloidal carbohydrates.

The integrity of the lymphatic system is essential to the frog, because of its important circulatory function in returning protein to the blood.

The suggestions and kindly criticisms of Dr. Cecil K. Drinker have been invaluable to the writer during the course of this work.

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DIFFERENTIAL RESPONSE OF MALE AND FEMALE RING DOVES TO METABOLISM MEASUREMENT AT HIGHER AND LOWER TEMPERATURES

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Received for publication July 8, 1930

In recent studies on the "basal" or standard metabolism of animals many investigators have considered it practically essential to find and utilize that environmental temperature at which the animal will produce a *minimum* of heat. We earlier found (1), and have since confirmed the result, that the normal "basal" metabolism of the ring doves described in this paper can be still further reduced—beyond that attainable by temperature alone (approximately 30°C.)—simply by confining the birds in very small cages (1 cubic foot, or 28 liters). In three series of tests this close confinement, during only four or five weeks, lowered the former "basal" by 5 to 7 per cent. This same fact was earlier demonstrated by Lusk and Du Bois (2) on dogs, and by Mitchell and Haines (3) on fowls. It is well known that hypothyroidism, certain other disorders, prolonged fasting, undernutrition, certain drugs, etc., are other means of obtaining *abnormally* low rates of heat production in animals and man. The results described in this paper indicate that (high) temperature also belongs in this group of factors which yield *abnormally* low values.

We here report and fully establish—for the first time in any animal—a differential response of the sexes to metabolism measurement at different temperatures. Besides the wide usefulness and applicability of this fact it seems—in connection with other and above-mentioned facts—to indicate that the *minimum* values derived from birds at relatively high temperatures (30°C.) are obtained on the animal while in an abnormal or "non-physiological" state. Certainly a temperature of 20°C. is much nearer that in which these animals normally live, and to which they are adjusted, than is 30°C. Some objective evidence of injury at 30°C. is found. Again, the relative metabolism values obtained by us for male and female birds at the lower temperatures are those which seem elsewhere to be normal for the sexes (see discussion), whereas in the minimum values obtained after insulting these birds with the higher temperature this relationship is

definitely reversed. Because of other fairly consistent evidence that males have a higher metabolic rate than females—and Riddle (4) has obtained indirect evidence of this on the *embryos* of these same doves—this aspect of our data also suggests that our minimum values from higher temperatures are obtained on birds in a non-physiological condition.

MATERIAL AND PROCEDURE. All of the data presented here were obtained after two years of preliminary investigation of the various conditions or factors necessary for the reliable measurement of heat production in these animals. Those factors are described in an earlier publication (1). They include a 24-hour preparatory period in a cage permitting flight; this cage is kept at the same temperature as that at which the birds are to be measured 24 to 32 hours later. This means that birds measured at 30°C. *had remained at a constant temperature of 30°C. for this entire period;* also that, except for some days of summer, this temperature was higher than that in which the birds were normally living; and, without exception, it was the only occasion on which they lived for an equal period at a high *and constant* temperature. At the beginning of this 24-hour preparatory period these doves were hand-fed (when necessary) to bring the amount of grain in their crops uniformly to 5 grams, and to this grain 5 grams of water were added; on the following day they were given free access to water until 6 or 7 hours before actual measurement was begun. They then gave an R. Q. very near to 0.72 at the time of measurement. The measurements were made with technique previously described (5), but in a multiple apparatus *at night*, in completely darkened chambers. We earlier demonstrated the real importance of these several procedures. The measurements were made on sexually mature birds during all seasons and months of the year.

Autopsies, and complete records of reproduction, permit the elimination of diseased animals. The animals used belong to physiologically standardized races—standardized for thyroid and pituitary size—which Riddle (1), (6) has developed during the past 10 years. The known distinctions of these races have proved highly advantageous to this study, and such racial and hybrid differences make it necessary to divide and to tabulate the data in their present form. Each race is here a primary unit.

In an earlier publication (1) we presented some evidence concerning the effect of age upon heat production in doves and pigeons. Since that time we have accumulated a large amount of data on this point. Without attempting to supply the complete basis for our calculations here we can state as follows: In the sexually mature birds (5–36 mo.) used in this study the metabolism decreases approximately 0.5 per cent per month of increasing age. This value has been used in making correction for the age factor in the data tabulated in this paper.

Our results are calculated on a basis of body weight (150 g. per 4 hours) and not for surface area. The bird weights lie mostly between 140 and 170

grams. At present a really accurate basis for determining the surfaces of these birds is not available and until it is obtained we prefer not to introduce a discrepancy into an expression of our results. Though the values as given do not permit a fair comparison with other animals, they are quite satisfactory for the purposes of the present paper.

EXPERIMENTAL RESULTS. Table 1 permits an examination of the more adequate and most decisive data. We adopted *five* as the minimum number of measurements necessary to permit an entry on this table. Other races or hybrids have been studied, but in less adequate numbers. In table 2 we supply the two groups of such additional data, consisting of 167 tests made at the two temperatures on the sexes of 10 races, and another series of tests on 6 races measured at 15°, 20° and 30°C.

The mean values obtained for the 16 races at the two temperatures are, of course, the essential items here. When measured at 30° it will be observed (table 1) that the females show the higher metabolism in *every one of the 16 races tested.* Measured at 20°, however, these same races (in large part, the same individual birds) show a higher metabolism in the males in 12 (race 62 when this value is corrected for age) of the 16 groups. Stated otherwise, females measured at 30°C. (and with values properly corrected for age) lose only 20.3 per cent of their metabolism at 20°; the males lose 28.1 per cent from their 20° value—a difference here of 7.8 per cent. A differential response of the sexes to measurement at 20° and at 30° C. is here unquestionable. This difference is fully corroborated by a considerable amount of additional data which will be presented only in summary form.

The 10 races summarized in table 2 yield values which are comparable in every respect with those obtained from the 15 races already described. In this case the metabolism of males is decreased 26.5 per cent in the 30° measurement; that of the females is decreased by only 19.3 per cent—a difference of 7.2 per cent. Again, the six races which were measured at 15° (35 tests), 20° (86 tests) and 30° (95 tests) indicate that the metabolism of the male decreases slightly more than that of the female—even in the region between 15° and 20°C.

Finally, among the common pigeons (birds belonging to a different zoölogical family from the ring doves) two groups of measurements show a differential response of the sexes to measurement at different temperatures. In one group (249 determinations on 7 races) the males measured at 30°C. give a metabolism 10.1 per cent, and the females only 6.9 per cent, below the 20° value. The second and much smaller group (only 110 determinations on 7 additional races) indicate a reduction of 12.0 per cent in the males and 10.4 per cent in the females. One further point concerning the figures for common pigeons is notable here. Though fewer data, and notably more variable metabolism data, have been obtained on the common pigeons than on ring doves the results with the pigeons show beyond ques-

TABLE 1

Comparison of metabolism of sexes in 16 races of ring doves measured at 20° and 30°C. (589 determinations)

RACE	BODY TEMPERATURE	MALES AT 20°C.			FEMALES AT 20°C.		
		Number of tests	Age	Calories*	Number of tests	Age	Calories*
	°C.		*months*			*months*	
63	42.3	8	21.4	3,756	5	23.4	3,595
62	42.2	11	28.3	3,574	7	14.0	3,620
N2	41.4	7	24.5	4,004	10	20.4	3,999
72	41.5	12	27.4	3,867	14	18.2	3,765
51	40.7	12	20.2	3,600	6	14.4	3,502
NA	42.1	10	17.2	3,826	12	17.9	3,844
B	41.9	5	22.4	3,716	7	17.9	3,446
36–62	41.9	10	16.5	3,679	13	16.1	3,891
36–72	41.1	6	14.5	3,494	9	14.4	3,663
72–62	42.0	8	13.4	4,296	8	14.1	4,082
63–62	41.9	5	16.1	4,157	7	12.7	4,079
72–OT	42.1	7	13.5	3,668	9	15.5	3,545
11–62	41.9	6	19.7	3,588	8	16.9	3,456
OT–O	41.5	6	25.3	3,548	7	18.4	3,777
N2–63	41.2	6	16.4	3,872	8	15.9	3,846
NA–72	42.0	7	14.5	4,158	6	15.7	3,871
Mean.....		(126)	19.5	3,806	(136)	16.6	3,749
Calories corrected for age (16.6) = *3861*					(16.6) = *3749*		
		At 30°C.			At 30°C.		
63	41.5	13	18.2	2,939	6	19.4	3,198
62	42.4	11	25.4	2,747	7	19.0	2,878
N2	41.7	17	20.2	3,092	7	16.0	3,159
72	41.5	10	24.3	2,656	15	18.6	3,047
51	40.9	12	17.6	2,898	10	13.7	3,122
NA	41.9	10	18.9	2,921	15	17.9	2,960
B	41.9	6	19.6	2,714	8	16.3	3,121
36–62	41.7	14	15.5	2,810	15	16.1	2,874
36–72	41.6	8	12.3	2,767	12	11.1	2,966
72–62	42.2	7	11.0	2,760	8	9.9	2,961
63–62	41.2	9	16.8	2,608	13	13.0	2,879
72–OT	41.6	10	14.1	2,621	9	15.5	2,869
11–62	41.7	10	14.4	2,708	11	16.6	3,054
OT–O	42.1	8	20.4	2,521	10	16.4	3,112
N2–63	41.4	9	16.5	2,843	13	16.8	3,061
NA–72	42.2	7	11.9	2,646	7	12.0	2,823
Mean.....		(161)	17.3	2,766	(166)	15.5	3,005
Calories corrected for age (16.6) = *2777* *2777* = *28.1* per cent below 20° value					(16.6) = *2989* *2989* = *20.3* per cent below 20° value		

* Small calories per 4 hours per 150 grams body weight.

tion that the metabolism of these animals *varies far less* with this 10°C. change of environmental temperature than does that of the ring doves. Male pigeons change by only 10.1 per cent (♀ = 6.9 per cent) whereas male doves suffer a change of 28.1 per cent (♀ = 20.3 per cent). Clearly, certain species and races—like a certain sex—are more affected by measurement at higher temperature than are other species and races.

Tables 1 and 2 contain important data on the relation of metabolism to sex. Since our measurements on this point are being greatly extended we need not consider this question at length here. It should be noted, however, that the present data make it quite certain that, when measured at

TABLE 2

Other data on metabolism in the sexes (ring doves) as related to environmental temperature; with percentage decline of metabolism per degree Centigrade (values corrected for age)

GROUP	SEX	NUMBER OF TESTS	CALORIES* AT:			PERCENTAGE DECREASE OF METABOLISM PER DEGREE BETWEEN	
			15°	20°	30°	15° to 20°	20° to 30°
16 races (table 1)	Male	287		3,861	2,777		2.81
	Female	302		3,749	2,989		2.03
10 races†	Male	73		3,862	2,840		2.65
	Female	94		3,756	3,031		1.93
6 races‡	Male	107	4,329	3,824	2,681	2.33	2.99
	Female	109	4,210	3,785	2,932	2.02	2.25

* Small calories per 4 hours per 150 grams body weight.

† Additional races for each of which fewer than five (average, 4+) measurements were made for one or the other sex at one or another temperature.

‡ Measured at 15°–20°–30°C.

30°C., the females of these ring dove races have the higher metabolism. On the other hand, the summary of the three series of measurements (table 2) uniformly indicates that the males have the higher metabolism when measurement is made at 20°. One series of measurements made at 15° results similarly—the male value here being higher by 2.83 per cent (♂ = 4329; ♀ 4210 cal.). At 20° the male value is higher, in the three cases, by 3.0, 2.8, and 1.6 per cent.

The above calculations are made on the basis of weight, not of surface. Since the males are somewhat larger (3 or 4 per cent), and their body surface therefore somewhat smaller relative to weight, it is evident that a calculation made on the basis of actual body surface would somewhat increase the

amount of this difference. Moreover, approximately one-fifth of the females were measured at or near the time of ovulation. It is probable that at this particular period the metabolism of the female is somewhat increased beyond that otherwise usual and normal for the female. This probability rests partly upon various blood and endocrine changes which Riddle (7) and co-workers have identified with this special period. These changes involve suprarenal hypertrophy, histological changes in thyroid, and very marked increase of blood sugar and blood calcium. Fowls also, in their egg-producing periods, according to Gerharz (8) and Mitchell and Haines (3), notably increase their basal heat production. Gerharz cites a case in which this increase was 44 per cent. Heat production values obtained at this period in our doves probably are abnormally high but they have here been recorded as typical or normal values for the female; their elimination would probably still further increase the excess of heat production shown by the males at 15° and 20°C.

There is the following objective evidence that a temperature of 30°C. (continuously sustained during 24 to 32 hours) sometimes or often places these doves and pigeons in a somewhat abnormal physiological state. Five birds have died during or immediately after subjection to a temperature of 29° to 33°C. apparently as the result, doubtless indirectly yet mainly as the result, of these temperatures. None has thus died in connection with a somewhat larger number of tests made at one or another lower temperature.

We shall not attempt here to present details of our evidence that 30°C. is approximately the "critical" temperature for ring doves. This will be fully considered in a later paper and after still further investigation of its variability in different races; Giaja (9) has already reported a critical temperature of 30° for this dove. It is already evident—in part, from the data of table 1—that this critical temperature is not precisely the same for the two sexes, nor for all the ring dove races, nor for any of these for summer and winter seasons, though in general these values fall more or less around 30°C. The data of table 1 would permit us to show specifically that the metabolism of certain races is more affected by high temperature than is that of other races. Also that certain races are consistently characterized by an unusual body temperature (e.g., race 51). These conditions are to be expected in this material since, as already noted, Riddle has succeeded in establishing several of these races on the basis of characteristically large or small size of pituitary and thyroid. We wish merely to note the presence of these differences without further discussing them in this paper.

COMMENT. It could be assumed, quite in accord with the usual view, that the present results indicate a greater stimulation of the metabolism of the male than of the female by lower environmental temperatures (20° to 15°) and not a greater depression of the male metabolism by an insultingly

high temperature (30°). We reject this way of regarding this demonstration of a differential response of the sexes, because of the various considerations raised in our introductory statement and of other facts discussed in the following paragraphs.

We conclude that many of the ring doves studied by us—taken at all seasons and previously subjected to a constant temperature of 30°C. for 24 to 30 hours—are in a "non-physiological" condition. We do not say they are pathological. But most of these animals (i.e., during most months of the year) are physiologically adjusted to a lower temperature and to a temperature which fluctuates slightly or considerably. We think of the 30° temperature as suddenly forcing a heavy burden upon the water regulatory mechanism at a time when the thyroid and other mechanisms for maintaining heat production (also quantity of food or energy intake) have not had sufficient time for their usual and normal abatement. Such a period of stress is markedly unusual and thus essentially abnormal; it can be considered physiological only in the sense that the organism's reaction against infection or invading disease is physiological—and both of these types of stress should be considered abnormal in a study of standard metabolism.

We do not suggest that *males* only are "among the many ring doves put in a non-physiological condition by subjection to a temperature of 30°." Some females are undoubtedly affected. One female "scraggly" common pigeon—a defectively feathered race which uniformly shows a very high rate of heat production—is among the five birds which died apparently as a result, however indirect, of subjection to this temperature. We further and specially note that the "critical" temperature for females may be slightly lower than that for males—and slightly lower for both sexes in some races and at some seasons than in others. But we face the definite fact that when measured at 30°—an *unusual* environmental temperature and one which is identical with or close to the "critical" temperature—the metabolism of the female has reacted less, and the male more, to the change from more *usual* environmental temperatures (20° to 15°). The actual metabolism of the female at this higher and *unusual* temperature, in relation to that of the male, is the reverse of that found under measurement at *usual* temperatures.

In a recent and important paper dealing with experiments on the adaptation of rats to air of reduced cooling power Sundstroem (10) finds that the "rats suffered a great deal from discomfort" during their first days in such a climate. Moreover, he obtained indications that female rats show less mortality than do males when both are exposed to air of low cooling power. These findings notably increase the probability that our very definite results (on heat production) with doves and pigeons will prove applicable also to mammals. Kayser (11) measured the heat production of some common pigeons (sex not stated) at 30°. He reports a decrease of 29.4 per cent as

the result of keeping a very few birds at 28° (in an incubator?) for a few weeks. But the higher value, on which this calculation was based, was obtained by measurement during daytime while the lower value was obtained at night. Benedict and Riddle have shown (1) that such measures are by no means comparable—the night measures giving distinctly lower values in common pigeons. It is further probable that much of the "reduction" obtained by Kayser is ascribable to a close laboratory confinement (inactivity) of his birds while kept at 28°, and perhaps partly to season. His relatively few measurements are not described sufficiently to permit a definite interpretation of his results.

Our studies on heat production in ring doves have thus already pointed out two conditions which reduce the metabolism below the point at which it can properly be called normal or standard (basal); and at least one of these conditions depresses the metabolism of the male more than that of the female. The first of these conditions is enforced inactivity, or confinement in very small cages (Benedict and Riddle, 1; and unpublished data); the second is high temperature. The confinement in small cages almost certainly induces an abnormal or non-physiological state in these animals. Aside from other general evidence Riddle and Honeywell (12) demonstrated that this treatment in pigeons causes the normal amount of sugar in the blood to decrease by nearly 30 per cent. In the introductory statement we noted that dogs and fowls have earlier been shown by others to have a lower "basal" heat production after confinement in the laboratory.

We have cited the death of five birds as evidence that high temperatures also place these birds in a non-physiological condition. If Riddle (13, 14) is correct in his insistence (from other studies on doves and pigeons) that metabolic rate bears a *primary*, not a secondary, relation to the differentiation of sex, and that the male sex can develop only under the relatively higher oxidation rate, then the birds as measured by us at 30°C. would certainly be in an abnormal state—since the females then show a definitely higher metabolism. On the basis of these and other facts one must doubt that the enforcement of a minimum metabolism is, in itself, of as little consequence to the male organism as to that of the female. Again, in all cases of better studied animals—fowl (3), (15) rat (16) and man (17), (18), (19)—the male sex has been found to have the higher metabolism. In addition, much evidence as been obtained from measurements and methods other than those of calorimetry which strongly support the conclusion that a higher rate of tissue oxidations is normally found in the males of the numerous plants and animals thus investigated (13).

Apart from any considerations concerning the problem of the fundamental nature of sexuality, the *fact* that the sexes among doves and pigeons show—in their normal metabolism—a differential response to environmental temperature would appear to be of far-reaching importance. It

seriously questions the "physiological" status of the animal driven by external heat to the production of a minimum of heat in metabolism measurements. It would seem to require an extensive reconsideration of the question of the temperature at which the normal heat production of any animal is to be measured. In experimental biology generally it cautions that males and females may be subjected to quite the same high environmental temperature without being equally insulted by it. In growth, vitamine and other nutritional studies—where food consumption, growth and metabolic rate are of chief interest—this fact probably has a wide and hitherto unsuspected application. In the case of highly accurate measurement very different results with the two sexes may be obtained merely through keeping the animals at, or temporarily subjecting them to, a higher or a lower environmental temperature.

SUMMARY

From 589 measurements made at 20° and 30°C., on males and females of 16 standardized races of ring doves it is definitely established that the metabolism of the male suffers a greater decrease with increase of external temperature than does that of the female. Between these limits the decrease in males is 28.1 per cent, in females 20.3 per cent. These values are further and fully confirmed by 167 measurements on 10 additional races, and by still other data.

Under extreme (and abnormal) depression of the metabolism by a temperature of 30°C. the metabolism of the females is higher (7.1 per cent) than that of the males. At 20°C. the measurements of all the three large racial groups indicate a higher normal metabolism in the male (3+ per cent). Additional but preliminary measurements made at 15°C. similarly indicate a higher metabolism in males when measured at this temperature.

The heat production of certain species and races—like that of the male sex—is much more depressed by a high external temperature than is that of other species or races. All races of common pigeons studied by us are much less affected than are the ring doves. Some races of ring doves—all of which races have been established on the basis of size of thyroid and pituitary—are much less depressed than other ring dove races.

Measurement at these temperatures was preceded during 24 hours by maintenance at the same temperature, and with all the technique earlier found necessary to the accurate measurement of heat production in these animals.

The measurements made at 30°C.—which is approximately the temperature that yields a *minimum* metabolism but with this varying somewhat for sex, race and season—supply evidence that such minimum values are obtained on birds in a non-physiological state. There is already some evidence that this applies to still other animals.

This differential response of the normal metabolism of the sexes to temperature is of much consequence not only in metabolism studies, but in growth, vitamine and nutritional research, and in experimental biology generally. In accurate measurements very different results with the two sexes may be obtained merely through keeping the animals at, or temporarily subjecting them to, a high or low environmental temperature.

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THE RELATION BETWEEN CONTRACTION FREQUENCY AND LACTATE ACCUMULATION, AND ITS BEARING UPON THE ECONOMY OF TENSION PRODUCTION AND MAINTENANCE IN STRIATED MUSCLE

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Received for publication May 13, 1930

I. THE DEPENDENCE OF LACTIC ACID PRODUCTION IN A CONTRACTION UPON THE ELAPSED TIME SINCE THE LAST CONTRACTION. It was found by Starling and Visscher (1927) that the oxygen consumption per contraction of the heart in the heart-lung preparation decreases with an increase in the heart rate. Hartree and Hill (1921a) had shown that the heat produced in contraction depends upon the interval between contractions. In the light of the current conception, based upon the work of Fletcher and Hopkins (1906), and others, particularly Meyerhof (1920), that the magnitude of the oxygen consumption in the recovery process is a function of the magnitude of the lactic acid concentration, it becomes of interest to know whether the lactic acid production in contraction changes with varying frequencies in a similar way.

Method. The production of lactic acid in contraction in relation to the interval between contractions has been investigated by comparing the lactate concentration in one gastrocnemius muscle of a frog after stimulation at a given frequency with that of the other gastrocnemius treated identically and stimulated the same number of times but at another frequency. The frogs used (Rana pipiens and Rana catesbiana) were precooled to bring the resting lactic acid level to a constant figure, and the animals were killed, transecting at one stroke the urostyle, the ilii, and the sciatic nerves about a centimeter caudal to the sacral vertebra, and immediately thereafter transecting the brain stem and pithing the brain. The sciatic nerves were cut before cord injury in order to obtain similar resting lactate levels on the two sides. Extreme care was observed in subsequent dissection and isolation of the gastrocnemius nerve-muscle preparation and if adventitious contractions occurred the preparation was discarded. The two gastrocnemii were prepared and placed in Ringer's fluid at the temperature at which the experiment was to be carried out. After attachment of the muscle to the isometric lever, a determined number

of maximal shocks was delivered to its nerve at a determined frequency. A mechanical record was made of the tension set up and maintained by the muscle during each contraction. The muscle support was so constructed that the muscle and the support could be rapidly lowered into a cylindrical Dewar flask filled with liquid oxygen. The muscles were weighed to the nearest 0.01 gram, and, while still frozen, minced and ground in sand and a known volume of 10 per cent sodium tungstate. After thorough grinding,

TABLE 1

Lactic acid production in opposite gastrocnemii of Rana pipiens treated alike except for frequency of stimulation

Temperature 22°C.

FROG NUMBER	NUMBER OF STIMULI	FREQUENCY OF STIMULATION 1/SECOND		FREQUENCY OF STIMULATION 6/SECOND		INCREASED L.A. PRODUCED AT LOWER FREQUENCY
		Post-stim. level of L.A.	L.A. liberated per stimulus	Post-stim. level of L.A.	L.A. liberated per stimulus	
		mgm. per cent	*mgm./100 grams*	*mgm. per cent*	*mgm./100 grams*	*mgm./100 grams muscle*
1	240	340	1.24	174	0.55	166
2	240	295	1.06	179	0.57	120
3	240	295	1.06	156	0.48	140
4	210	345	1.44	225	0.87	120
5	240	375	1.42	212	0.71	163
6	210	325	1.34	177	0.64	148
7	210	365	1.54	180	0.66	185
8	210	328	1.36	177	0.64	151
9	240	307	1.10	158	0.48	149
10	240	300	1.08	124	0.34	176
Mean..		328	1.26	176	0.59	152
Mean deviation.		23	0.16	19	0.14	17

In calculating L.A. liberated per stimulus the average resting level 0.041 per cent is utilized. An error is introduced by this procedure which is probably not greater in any instance than the maximum observed deviation from the average, namely, +0.019 mgm. or −0.010 mgm. per 100 grams muscle. In the average this error, which is random, tends to cancel out.

seven volumes of water and a volume of $2/3 N H_2SO_4$ were added, and later distilled water to make the dilution 1:20. Following the suggestion of Ronzoni and Wallen-Lawrence (1927) the dilution was made greater than the usual 1:10 in order to give a smaller percentage retention of lactate in the precipitate. The determination of the lactate was made in the filtrate following the method of Friedeman, Cotonio, and Shaffer (1927) as modified by Davenport and Cotonio (1927).

Results. Table 1 presents typical results of a series of measurements of the lactic acid production in pairs of gastrocnemii treated exactly alike

except for frequency of stimulation. Twenty-five such experiments have been performed. One muscle of a pair was stimulated at 1 per second. There is uniformly a greater lactic acid content in the muscles stimulated a given number of times at lower frequency.

In order to arrive at the lactic acid production per contraction it is necessary to know the so-called "resting level," more properly the initial

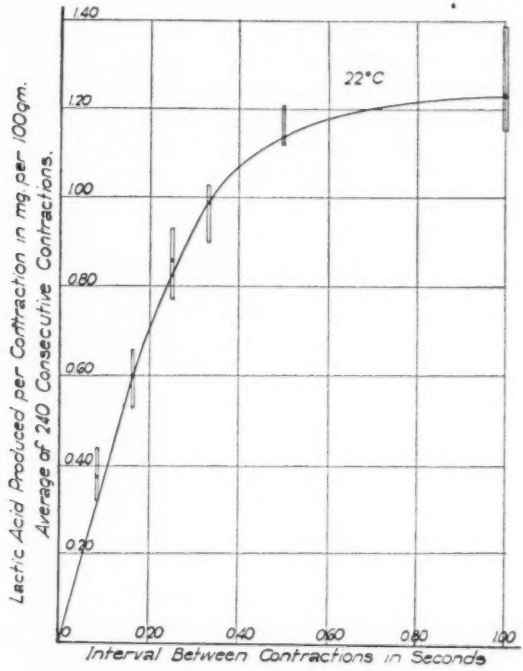

Fig. 1. Lactic acid production in relation to frequency of stimulation. Each point represents the mean value and the open block includes the median 50 per cent of all values at the particular frequency.

level. This value has been found to average 0.041 per cent and to be sufficiently uniform to permit the use of the average figure in subtracting from the observed lactic acid content of a stimulated muscle to arrive at the true lactic acid production. Making such a subtraction, and calculating the lactic acid produced per contraction, one finds on an average 1.25 mgm. liberated per 100 grams muscle when the interval between stimuli is 1 second and the total number of consecutive contractions is 240 or thereabouts. When the interval is 0.17 second the amount produced is almost exactly half as great. This observation has been controlled by

stimulating both muscles of pairs at the same rates, in which case it is found in nine experiments that the two muscles have lactic acid content agreeing within 10 per cent, which may be taken as the limit of accuracy of the methods.

To demonstrate further the dependence of lactic acid formation upon the interval between contractions other intervals were used. In figure 1 the results are given graphically. The solid points in the open bars represent the average of all determinations at a given frequency, while the open bar itself includes the median 50 per cent of values at that frequency. Considering the complexity of the procedure, the regularity of the observed increase in lactic acid production with the interval between contractions is satisfactory. The points at 0.085, 0.25, and 0.50 second are less significant than the other three because they are the result of fewer experiments. At each of the points 0.17, 0.33, and 1.00 second, twenty or more observations have been made.

The effect of temperature upon the rate of lactic acid production per stimulus has been investigated in twenty-five muscles. The rate at 6°C., 16°C., and 26°C., at contraction intervals of 0.33 and 1.00 second, has been determined and the results show that the rate at both frequencies increases with a rise in temperature. The number of experiments at each temperature is too small to allow us to be quite sure of the accuracy of the mean, without which analysis of a temperature coefficient is not significant.

The limiting effect of elapsed time since a preceding contraction upon the lactate production in any new contraction is related, in all probability, not to the rate of production of "precursor," but to the rate of neutralization of free lactic acid. Indirect evidence of several sorts makes this conclusion probable.

The fact that a partial tetanus is encountered at a stimulation frequency of 6 per second indicates that the tension producing mechanism has not recovered from the preceding contraction before a new one occurs. In the light of our current notions of the factors responsible for tension changes in contraction, this seems to show that the free lactic acid has not been removed before the next contraction occurs. If the concentration of free lactic acid in the contractile elements determines the tension set up, then it is apparent that a maintenance of tension indicates incomplete removal of lactic acid. If lactic acid concentration has nothing whatever to do with tension changes, as Bethe (1922), Embden (1925), and others contend, then, of course, there are no grounds for believing that the continued tension after contraction has any bearing upon lactic acid concentration. Evidence then would have to be sought from other sources. But the preponderance of evidence at the present time seems to indicate that there is a quantitative relationship between lactic acid production and tension production, strong additional evidence for which is given in the

next section of this paper. It would be an error in logic to assume a causal connection between lactic acid production and tension production simply on the basis of the observed coincidence in magnitude and time of two processes, but it is obvious that they must be very closely interrelated. In normal muscle contraction one never finds tension set up without a production of lactic acid in amount commensurate with the tension. This is true even under apparently adverse conditions, such as low glycogen content, anaerobic fatigue, etc. (Smith and Visscher, 1930), although the relationship does not hold when the tension-producing machine has been altered by anesthetics (Meyerhof, 1921) or by accumulation of waste products of prolonged metabolism after isolation from the waste disposal mechanism in the isolated heart-lung preparation (Starling and Visscher, 1927).

When the tension maintained by muscles stimulated at short intervals is measured it is found that the tension is always approximately as high as in contraction at longer intervals. If there were a shortage of "lactic acid precursor," or too slow a rate of production of lactic acid itself, there should be less tension maintained when contraction occurs at high frequencies. It seems impossible to escape the deduction from this fact that the slowest reaction in the process is the removal of the tension-producing agent in the anaerobic recovery.

II. THE ECONOMY OF TENSION DEVELOPMENT AND TENSION MAINTENANCE IN RELATION TO CONTRACTION FREQUENCY AND THE RATE OF ANAEROBIC RECOVERY. In a series of isometric twitches in unfatigued muscle the tension developed is found to be related to the lactate accumulation and to the heat production in a simple way. Hartree and Hill (1921b) showed that $\frac{TL}{H}$ = Constant, where T is the maximum tension set up, in kilograms, L is the muscle length, in centimeters, and H is the heat production. Meyerhof (1921) showed that under similar conditions $\frac{TL}{L.A.}$ = Constant, where the other symbols are the same as above and $L.A.$ represents milligrams of lactate per 100 grams of muscle.

In studying the coefficient of tension development and lactate accumulation at differing frequencies of stimulation it is found that the total tension *maintained* per unit of lactate formed is very much greater with rapid stimulation than with slow. In table 2 appear typical results of measurements of the isometric tension coefficient of lactic acid at stimulation rates of 1 and 6 per second. The data given in this table are from the experiments for which the lactic acid figures are given in table 1. In the columns headed Total Tension Coefficient are tabulated the values of $\frac{TL}{L.A.} = C$, where T represents the sum of the maximum tensions arrived at in each

contraction over the period in which the lactate accumulation is measured. In the column of Developed Tension Coefficients the calculations are made for the same experiments letting T equal new tension developed in each contraction. Thus, in the latter case, if contraction begins at a tension of 500 grams and the highest tension set up is 750 grams, T = 250 grams, whereas for the calculations of the Total Tension Coefficient T = 750 grams.

C_t, the coefficient relating lactic acid production to the total tension maintained is 121.8 as the average of 35 observations at 1 stimulus per second. C_d, the coefficient relating lactic acid to new tension developed, is 81.8 on the average at that rate of stimulation. The coefficient C_t is higher than C_d because of the occurrence of contracture, and because a small initial tension, 20 to 50 grams, is imposed in order to fix the muscle

TABLE 2

The developed and total tension coefficients of lactic acid in relation to frequency of contraction

FROG NUMBER	1 STIMULUS PER SECOND		6 STIMULI PER SECOND	
	C_t—total tension coefficient	C_d—developed tension coefficient	C_t—total tension coefficient	C_d—developed tension coefficient
1	151	85	371	86
2	147	94	329	98
3	146	70	498	50
4	109	96	242	62
5	110	94	359	52
6	158	70	522	49
7	96	68	375	76
8	75	67	197	97
9	140	89	637	33
10	153	63	790	62

at a definite length. At a stimulation frequency of 6 per second the coefficient C_t averages 467 in 15 observations while C_d is 69.8. Thus C_t differs by several hundred per cent at the two frequencies, while C_d, relating developed tension to lactic acid formation, is somewhat less than 20 per cent more at 1 per second than at 6 per second.

The developed tension is obviously more closely related to lactic acid formation than is the total tension if lactic acid liberation is causative to the development of tension in contraction. The fact that C_d is approximately 20 per cent greater at 1 per second that at the 6 per second rate of stimulation may be due to the fact that there is incomplete relaxation at the higher frequency, and each contraction begins at a higher tension level. This may mean that it is more expensive to build up tension from high than from low initial tensions. This is found to be the case when high initial tensions are imposed by stretching. An example of an experiment in

which high initial tension is mechanically imposed is given in table 3. The developed tension coefficient is very low, in comparison with the control in which the initial tension was small, while the coefficient of total maintained tension is much greater in the case of the high initial tension than of the low. This fact does not prove, however, that tension development at the higher tension levels would be any more expensive when the high initial tension is due to the presence of unneutralized lactic acid.

The total tension maintained in each contraction per unit of lactic acid liberated is much larger at the rapid rates of stimulation, probably because the free lactic acid concentration in the active portions of the muscle has not had time to fall to zero since the last contraction. The magnitude of C_t depends primarily upon the rate of removal of lactic acid. Thus the economy of tension maintenance at rapid rates of stimulation is greater than at slow rates because the free lactic acid of a previous contraction is still exerting its effect upon the tension producing mechanism when the new contraction occurs.

TABLE 3

Dependence of isometric tension coefficient upon initial tension altered by changes in initial length

INITIAL TENSION	TOTAL TENSION SET UP	DEVELOPED TENSION COEFFICIENT	TOTAL TENSION COEFFICIENT
grams	*kgm.*		
500	13.0	31	208
30	35.0	86	146

The fact that free lactic acid is neutralized at a measurable velocity, and not instantaneously, is of advantage to the muscle in that it makes tension maintenance more economical than it could be if new lactic acid would have to be formed for all of the tension of each contraction. The efficiency of holding tension in a tetanus varies inversely with the rate at which lactic acid is removed; in this one finds explanation for the observation of Hartree and Hill (1921) that the economy of tension maintenance is greater at lower temperatures. If the rate of acid neutralization is slower at lower temperatures, less new lactic acid need be formed to set up a given tension. In our own results we have found the total tension coefficient of lactate accumulation to be higher at low temperatures, falling in line with the view we have put forward.

The tetanus of part of the fibres is a more economical method of maintaining tension in a muscle group than would be the successive single twitches of alternate fibers, because, in the successive contractions of the same fiber, lactic acid carried over from previous contractions is of use.

III. THE SIGNIFICANCE OF LACTIC ACID REMOVAL IN THE SO-CALLED

FATIGUE CONTRACTURE. When a muscle is maximally stimulated once per second through its nerve it shows the well-known failure of relaxation after a certain number of stimulations, the number varying with different muscles. In the light of the interpretation we have presented in this paper the failure of relaxation in muscle after repeated stimulation, even at slower rates, is a result of incomplete neutralization of free lactic acid in the anaerobic recovery. It occurs when the store of base has been depleted to such a low level that the rate of neutralization of free lactic acid is slowed appreciably by the low concentration of buffer.

This view of fatigue contracture will perhaps explain the extraordinary rapidity of occurrence of rigor mortis in mammalian muscle after severe insulin convulsions, observed by Hoet and Marks (1926). They showed, and it has been shown in the frog by Olmsted and Coulthard (1928) and by the authors (Smith and Visscher, 1930) that the glycogen content of the muscle is frequently very nearly zero following convulsions as a result of the repeated contractions. The frequently repeated contractions have also depleted the store of available base to nearly zero and any large acid production will remain unneutralized.

SUMMARY

1. The lactic acid produced per contraction in the frog's gastrocnemius is inversely proportional to the frequency of stimulation.

2. It is found that the lactic acid coefficient of developed tension is a more constant factor than is the coefficient of total maintained tension. The latter is very different at various frequencies of contraction, whereas the former is nearly a constant.

3. The total tension coefficient is a measure of economy of tension maintenance in contraction, and indicates a marked increase in efficiency of tension maintenance at higher frequencies.

4. The slow rate of removal of lactic acid is of advantage to the organism in those muscles where the maintenance of tension is an important function.

5. Fatigue contracture appears, in the light of the experiments reported, to be due to the depletion to a very low level of the store of base for neutralizing free lactic acid.

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ON THE SOURCE OF ENERGY FOR ANAEROBIC CONTRACTION IN GLYCOGEN-POOR MUSCLE

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Received for publication May 13, 1930

The concept that the energy of anaerobic contraction in muscle arises from the breakdown of glycogen to lactic acid and the associated reactions has been seriously questioned by Olmsted and Harvey (1927) and Olmsted and Coulthard (1928) who have made observations upon muscle with very low glycogen stores which lead them to conclude that in such muscles the energy for contraction must come from another source than the formation of lactic acid from glycogen and the associated reactions, and that some lactic acid arises from an unknown source, not glycogen. Meyerhof and Himwich (1924) studied the carbohydrate balance in glycogen-poor muscle from the rat but found, however, no evidence of lactic acid formation from non-carbohydrate sources. They made no quantitative observations upon the sources of energy for contraction on glycogen-poor muscle.

The conditions of the experiments of Olmsted and Harvey consisted of a study of the contraction of frog's muscle in situ in the virtually intact animal. In these experiments the muscles of frogs in which convulsions had been induced by over-dosage of insulin behaved much like the normal although on analysis of the opposite resting limb very little glycogen was found to be present. The influence of the other organs and an intact circulation make it impossible, however, to analyse such a situation very closely. Olmsted and Coulthard (1928), on the other hand, excised muscles from frogs in severe insulin convulsions and subjected these excised muscles to stimulation to fatigue. In such muscles they reported a fatigue curve closely resembling the normal, and a production of lactic acid not accounted for by the disappearance of carbohydrate.

These findings, if corroborated, would necessitate a revision of current notions as to the character of the chemical reactions in contraction. We have therefore re-investigated the problem in an attempt to settle the questions these workers have raised.

METHOD. Rana pipiens and Rana catesbiana were used in the following experiments. In order to obtain sufficient muscle for an accurate analysis in the case of the small frogs, the entire hind limb was used, thigh and

gastrocnemius muscles being finely minced and carefully mixed after having been frozen in liquid air to prevent injury production of lactic acid and to halt metabolic changes rapidly. Control determinations showed that the mixing was sufficiently thorough to offset the difference in glycogen content of thigh and gastrocnemius muscles. In the case of the large bull frogs the gastrocnemius muscles only were used. Insulin was administered to the experimental animals by injection into the dorsal lymph sac. Twenty-four hours following the first injection of 5 units most of the animals were hypersensitive but not in convulsions. A second 5 units was then injected and the animal taken at the height of the convulsions which followed. If necessary to induce the convulsions, a third injection was made. The frogs were killed by cutting transversely across the spinal column at the sciatic plexuses, and pithing immediately thereafter. The two hind limbs or the gastrocnemii were then dissected out on an ice-cooled glass plate. One was frozen in liquid air; the other was prepared for stimulation.

Control experiments were made to determine whether the simple act of dissection resulted in a production of lactic acid above the resting level. The resting muscles of frogs A and B, table 1, for example, were subjected to such manipulation and it has been found uniformly that the necessary manipulation, carefully carried out, results in no measurable increase in lactate. The muscles for study were attached either to an isotonic or to a tension lever and stimulated with induction shocks two per second until fatigued. The stimulation was indirect, through the sciatic nerve in the case of the large frogs' gastrocnemii and direct with the small frogs' hind limbs. After stimulation to fatigue the muscles were immediately immersed in liquid air. The muscle was finely minced and mixed while completely frozen. Half of the mixed muscle was taken for determination of lactic acid, and half was placed in an equal volume of 30 per cent KOH at 100°C. for the determination of glycogen.

The Friedemann, Shaffer and Cotonio (1927) method was used for the determination of lactic acid. The apparatus used was that recommended by Davenport and Cotonio (1927). Analyses of standard zinc lactate solutions show a recovery of 96 $\pm$ 3 per cent in 40 serial trials with quantities of 0.2 to 1.0 mgm. The zinc lactate used was prepared in this laboratory from zinc carbonate and lactic acid of C. P. grade and was washed and recrystallized several times. The lactate was dried at 110°C. to constant weight previous to use. Upon ashing, the sample showed a purity of 99.9 per cent.

The protein-free filtrates for the lactic acid determinations were prepared as described by Visscher and Smith (1930).

A modified Pflüger method was used for the determination of glycogen. The changes in general followed the suggestions of Evans (1926), except that repeated washing was avoided in the later experiments.

TABLE 1

Glycogen and lactic acid in resting and fatigued muscles

FROG	GLYCOGEN			LACTIC ACID			NOTES
	Resting	Fatigued	Loss	Resting	Fatigued	Gain	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
L	0.725	0.458	0.267	0.056	0.297	0.241	Winter grass frogs normal
M	0.880	0.690	0.190	0.030	0.217	0.187	Winter grass frogs normal
N	0.783	0.498	0.285	0.066	0.354	0.288	Winter grass frogs normal
O	Lost	Lost		0.060	0.262	0.201	Winter grass frogs normal
A	0.236	0.064	0.172	0.048	0.265	0.217	Spring grass frogs normal
B	0.465	0.285	0.180	0.045	0.265	0.220	Spring grass frogs normal
C	0.746	Lost		0.060	0.290	0.230	Spring grass frogs normal
D	0.063	0.000	0.063	0.055	0.121	0.066	Spring grass frogs normal
E	0.310	0.036	0.274	0.031	0.263	0.232	Spring grass frogs normal
F	0.454	0.250	0.204	0.035	0.240	0.205	Spring grass frogs normal
G	0.509	0.203	0.306	0.039	0.326	0.287	Spring grass frogs normal
J	0.319	0.142	0.177	0.043	0.228	0.185	Spring grass frogs normal
5	0.485	0.168	0.317	0.034	0.329	0.295	Spring bull frogs normal
6	0.561	0.471	0.090	0.027	0.124	0.097	Spring bull frogs normal
7	0.477	0.276	0.201	0.028	0.171	0.143	Spring bull frogs normal
P	0.330	0.063	0.267	0.102	0.359	0.257	W. G. F.—mild convulsions
Q	0.536	0.282	0.254	0.091	0.341	0.250	W. G. F.—mild convulsions
R	0.323	0.088	0.235	0.104	0.284	0.180	W. G. F.—mild convulsions
S	0.704	0.473	0.231	0.091	0.320	0.229	W. G. F.—mild convulsions
U	0.647	0.412	0.235	0.151	0.320	0.169	W. G. F.—mild convulsions
Bi	0.000	0.000	0.000	0.027	0.068	0.041	W. G. F.—severe convulsions
Ci	0.216	0.000	0.216	0.046	0.234	0.188	W. G. F.—severe convulsions
Di	0.093	0.000	0.093	0.073	0.178	0.105	W. G. F.—severe convulsions
Ei	0.171	Lost		0.030	0.189	0.159	W. G. F.—severe convulsions
Fi	0.080	0.000	0.80	0.035	0.156	0.121	W. G. F.—severe convulsions
Hi	0.229	0.000	0.229	0.033	0.258	0.225	W. G. F.—severe convulsions
Ii	0.000	0.000	0.000	0.028	0.076	0.048	W. G. F.—severe convulsions
Ji	0.079	0.000	0.079	0.130	0.220	0.090	W. G. F.—severe convulsions
Ki	0.000	0.000	0.000	0.034	0.041	0.007	S. B. F.—severe convulsions
1	0.329	0.205	0.122	0.058	0.198	0.140	S. B. F.—severe convulsions
2	0.061	0.000	0.061	0.028	0.047	0.019	S. B. F.—severe convulsions
3	0.204	0.100	0.104	0.079	0.194	0.115	S. B. F.—severe convulsions
4	0.182	0.161	0.021	0.030	0.087	0.057	S. B. F.—severe convulsions
Average for all normals.....			0.210			0.205	
Average for all after insulin convulsions.....			0.124			0.126	

An average hind limb yielded about 3 grams of muscle, a sufficient quantity for accurate analysis. The gastrocnemii of the bull frogs ordinarily weighed approximately 10 grams.

EXPERIMENTAL RESULTS. *1. The correspondence between glycogen disappearance and lactate production in insulinized frogs, compared with the normal.* In a series of 48 grass and bull frogs, the procedure of Olmsted and Coulthard has been duplicated as nearly as was possible. In one series of 15 large bull frogs the procedure has been altered to allow the measurement of developed tension as well as glycogen and lactic acid.

The general result of these experiments is that there is no lactic acid production, even in glycogen-poor muscles, which is not balanced, within the limits of experimental error, by a decrease in glycogen. In the normal frogs, table 1, the average increase in lactic acid in stimulation to fatigue

TABLE 2

Recovery of added glycogen from muscle rendered glycogen-poor by standing at 37°C. for 24 hours

WEIGHT OF MUSCLE TISSUE	GLYCOGEN ADDED	GLYCOGEN ADDED	GLYCOGEN RECOVERED	RECOVERY
grams	*mgm.*	*mgm./100 gm.*	*mgm./100 gm.*	*per cent*
3.65	0.00	0.0	0.0	0
3.13	0.00	0.0	0.0	0
1.31	0.32	23.0	0.0	0
1.59	0.60	38.0	23.0	61
1.74	0.90	52.0	42.0	81
1.77	1.51	85.0	94.0	110
1.86	3.02	162.0	158.0	97
1.65	4.52	274.0	272.0	99

is 0.210 per cent and the average loss in glycogen is 0.205 per cent. In the muscles made glycogen-poor by insulin convulsions there is equally good correspondence, the average glycogen loss being 0.124 per cent and the lactate gain being 0.126 per cent. In the muscles with very low resting glycogen levels, that is, after severe convulsions, the measured glycogen loss is somewhat lower than the lactic acid gain. In fact we find, as did Olmsted and Coulthard, that occasionally muscles from insulinized frogs have concentrations of glycogen too small for analysis, in which there is still some lactic acid production. In these muscles the gain in lactic acid is of course greater than the *apparent* loss of glycogen. In view of the fact that the glycogen method is known to be subject to greater errors than is the determination of lactic acid in small quantities, we have investigated the recovery of glycogen added to glycogen-free muscle. If small quantities of glycogen are lost in analysis one would expect that the lactic acid production would be somewhat greater than the *measured* glycogen loss.

Muscle was made glycogen-free by mincing and allowing it to stand for 24 hours at 37°C. On addition of glycogen to this muscle in quantities varying from the equivalent of 0.023 to 0.274 per cent in the muscle, the recovery rises from 0 per cent at the lowest concentration to approximately 100 per cent at a concentration in the muscle of 0.085 per cent and above, as is indicated in table 2.

When one looks at the results of the balance experiments in the glycogen-poor muscles in the light of these results it is obvious that one could not expect to find complete correspondence between glycogen loss and lactic acid gain if the glycogen content is below 0.050 per cent. A difference of the order of magnitude of 0.050 per cent lactic acid is to be expected because of the inadequacy of the glycogen method at very low concentrations. It will be noted in table 1 that 0.050 per cent lactic acid is the maximum discrepancy to be found, and in general the figures are considerably closer. The important points are, however, that in no case do we observe a lactic acid formation out of proportion to glycogen loss in insulinized muscle where the muscle glycogen has not reached a level below which it is impossible to determine it accurately, and that when the glycogen does fall below that level the lactic acid production is never greater than that which could come from the indeterminable glycogen. Consequently it is unwarranted to interpret the small difference between lactic acid accumulation and glycogen loss observed at the low glycogen concentration as indicating a formation of lactic acid from sources other than glycogen.

In the insulin treated frogs the fatigue maximum of lactic acid is lower than in the normal. This is not due entirely to insufficiency of glycogen, since it is to be noted in cases where very considerable quantities of glycogen remain after complete fatigue. It seems probable in the light of Meyerhof's (1921) observation that the amount of available base limits the magnitude of lactate accumulation, that in the insulinized frogs the repeated convulsions have permitted loss of creatine phosphate or other buffer, by diffusion into the blood stream with the lactate, in that way lowering the fatigue maximum.

2. The isometric tension coefficient of lactic acid. Under normal circumstances in skeletal muscle it has been shown that there is a quantitative correspondence between lactic acid production and the tension set up. The tension set up in unit length of muscle per unit of lactic acid formed is a satisfactory constant. For muscles of various lengths this relation may be expressed as

$$\frac{TL}{L.A.} = C$$

where T is developed tension, L is length, $L.A.$ is lactic acid produced. If T is expressed in kilograms, L in centimeters and $L.A.$ in milligrams per

100 grams muscle, the quantity C for a strictly isometric contraction has an average value of 82 (Visscher and Smith, 1930). In order to determine directly whether or not any energy for contraction in the glycogen-poor muscle may come from some other source than the formation of lactic acid it is necessary to determine the tension coefficient of lactic acid in such muscles. If energy for contraction in glycogen-poor muscle comes from another source than the heat of formation of lactic acid and the reactions associated with it, the coefficient relating lactic acid production to developed tension should be altered. The value C should be increased if energy comes from another source.

TABLE 3

Resting and fatigue levels of glycogen and lactic acid, and tension coefficients, large bull frogs

FROG	GLYCOGEN			LACTIC ACID			DEVELOPED TENSION	DEVELOPED TENSION COEFFICIENT	TOTAL TENSION COEFFICIENT	NOTES
	Resting	Fatigued	Loss	Resting	Fatigued	Gain				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*kgm./100 grams muscle*			
1L	0.800	0.540	0.260	0.100	0.370	0.270	2,441	45	59	Normal
2L	0.685	0.447	0.238	0.050	0.270	0.220	1,180	32	41	Normal
3L	0.700	0.426	0.274	0.050	0.300	0.250	1,820	39	58	Normal
5L	0.505	0.251	0.254	0.052	0.336	0.284	1,440	31	60	Normal
6L	0.944	0.633	0.311	0.056	0.375	0.319	2,060	36	64	Normal
1A	0.423	0.305	0.118	0.130	0.278	0.148	766	31	55	Convulsions
2A	0.223	0.194	0.029	0.246	0.262	0.016	122	42	238	Convulsions
3A	0.368	0.276	0.092	0.124	0.264	0.140	1,128	40	49	Convulsions
4A	0.220	0.170	0.050	0.047	0.092	0.045	324	43	103	Convulsions
5A	0.455	0.174	0.271	0.049	0.356	0.307	2,600	48	62	Convulsions
6A	0.322	0.151	0.171	0.033	0.268	0.235	2,080	48	61	Convulsions

The tension set up by normal and by glycogen-poor muscle and the lactic acid production in each case have been measured. The tension lever employed in these experiments allowed a certain degree of shortening, therefore the contractions are not truly isometric and the developed tension coefficients obtained cannot be looked upon as strictly isometric tension constants. For the purpose of this experiment, however, the comparison of the normal with the glycogen-poor muscle requires, not specifically isometric conditions, but rather simply comparable conditions in the two series. There is no particular merit, in other words, in perfect isometricity for making this comparison.

Table 3 presents the results of studies on the tension developed in relation to the chemical changes. The coefficient of developed tension (for explanation of the significance of the Total Tension Coefficient, see Visscher and

Smith, 1930) is seen to be substantially the same in the normal and in the insulinized animals. The range of variation of the coefficient is from 31 to 45 in five normal, and from 31 to 48 in six insulinized frogs. If any large amount of energy in the insulin treated frogs had come from another source than lactic acid production it might be anticipated that the tension coefficient would rise to a high value. It would, certainly, unless there were a concomitant decrease in the efficiency of producing tension in the muscle, such as occurs in the presence of narcotics (Meyerhof, 1921).

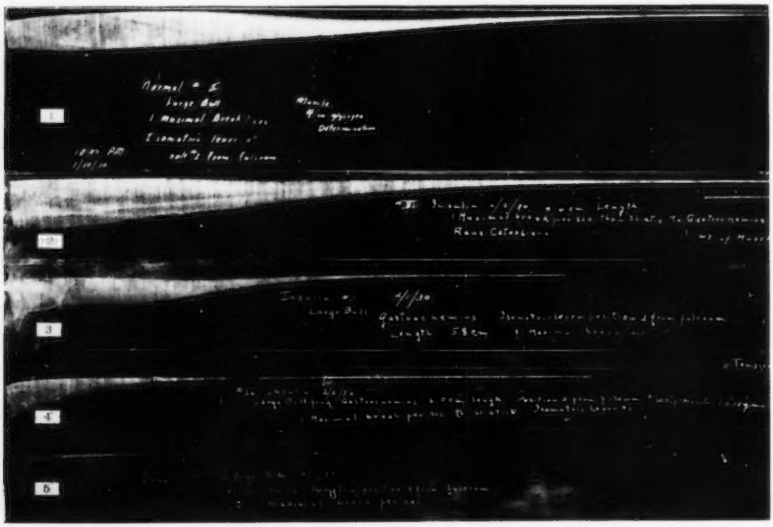

Fig. 1 Records of isometric contraction to fatigue of normal and glycogen-poor muscle. Tracing 1 is that of a normal muscle. Numbers 2 to 5 are those from insulinized frogs, showing progressively decreasing lactate productions. In 1 the lactate production was 0.220 per cent; in 2, 0.235 per cent; in 3, 0.148 per cent; in 4, 0.045 per cent; and in 5, 0.016 per cent. The developed tension coefficients may be found in table 3.

In figure 1 are shown the records of tension production in normal and insulinized muscle. These tension records show in a striking way that as lactic acid production falls off the tension produced likewise falls. Since in contraction to fatigue in muscle 5A more than twenty times as much tension was set up as in 2A it constitutes perfect agreement that the coefficients of developed tension in the two agree within 15 per cent, a figure within the experimental error of the measurement of the small lactic acid production in muscle 2A. In this muscle one might have expected a coefficient several hundred per cent removed from that found

normally, if there were appreciable sources of energy for contraction from other sources. The constancy of the developed tension coefficient in muscles showing the extremes of glycogen content and lactic acid production, appears to be very strong presumptive evidence that the energy for the contraction process must always come from the heat of the reactions associated with lactic acid production.

In twenty-four experiments recording isotonic contractions we have found that the magnitude of the mechanical response is invariably proportional to the quantity of lactate formed from glycogen. This correspondence may in reality be found in the experiments of Olmsted and Coulthard (1928, p. 615) where one finds in insulinized frog 53b, the only case in which a mechanical record is given, that the glycogen loss is quite comparable to that occurring normally.

It is not to be inferred from the results reported here that muscle contraction under aerobic conditions must always utilize lactic acid or carbohydrate for oxidation in the restitution processes. The finding of Visscher (1928) and Visscher and Mulder (1930) that in the isolated heart-lung preparation with low concentrations of blood glucose as little as twenty per cent of the oxygen consumption can be accounted for on the basis of carbohydrate disappearance makes it seem very probable that, at least in cardiac muscle, the oxidative recovery process is largely at the expense of non-carbohydrate material when the glucose concentration is low. It is not at all improbable that such a condition may prevail in striated muscle, and that when there is a lack of free carbohydrate some other material is oxidized to provide the energy for aerobic recovery. The experiments reported in this paper demonstrate only that in glycogen-poor muscle the energy of anaerobic contraction can be accounted for quantitatively on the basis of that expected from the observed formation of lactate from glycogen.

SUMMARY

1. Within the limits of experimental error the lactate accumulation in anaerobic contraction to fatigue is entirely balanced by the loss in glycogen in glycogen-poor, as well as in glycogen-rich muscle.

2. The magnitude of the mechanical response in isotonic contractions bears a direct relation to the magnitude of the lactate production regardless of the glycogen content.

3. The quantitative relationship which exists between lactate formation and tension set up in normal skeletal muscle has been found to hold equally well in glycogen-poor muscle. Although the sum of developed tensions in the extreme cases differs by twenty times, the coefficient relating lactate accumulation to tension set up is constant, thus indicating that even under the extreme conditions the energy for the setting up of tension always comes from the same set of exothermic reactions.

4. From these studies there is no evidence that the energy for tension development in muscle poor in glycogen arises from a different source than in muscles rich in glycogen. The constancy of the developed tension coefficient of lactate at widely varying levels of glycogen content, moreover, lends strong support to the hypothesis that the reactions associated with the production of lactic acid yield the energy for the contraction process.

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THE EFFECT OF TEMPERATURE UPON CHRONAXIE AND RECOVERY PERIOD OF NERVE

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Received for publication June 21, 1930

The effect of cooling upon certain aspects of nerve function—e.g., rate of conduction, recovery period, chronaxie—has been studied by several investigators. Most of the researches have been concerned with one or other of these phenomena, and have been carried out primarily for the purpose of throwing light upon the general nature ("chemical" or "physical") of these processes. Thus while it has been found that cold prolongs them all, little attention seems to have been given to the question whether during cooling these items of nerve function bear a constant relation to each other.

Evidence that such a relation may exist between chronaxie and recovery period has been reported by the authors (1930). The sciatic-gastrocnemius preparation of the frog (*Rana pipiens*) was used in these experiments. The excised preparation was kept in a moist chamber throughout, the arrangement being such that while the temperature of the nerve could be varied from that of the room to 0°C., the muscle was always above 15°C. A period of ten minutes was allowed at each temperature point for establishment of equilibrium. The stimulating electrodes were applied in the centre of the cooled region. In the first series of experiments the influence of various temperatures upon the chronaxie was determined by means of a Lapicque chronaximeter. The duration of the recovery period was measured in another series of experiments, by the method of least interval for muscular summation. The two stimuli applied were of equal strength, just above the maximal threshold value at the moment.

The results of both sets of observations, obtained in 12 different experiments and on 12 different preparations, are represented in the accompanying figure. All the points can be arranged along a fairly uniform curve. To make this evident, it is necessary to plot the ordinates (time) of recovery period as 6 times those of chronaxie at any given temperature.

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This would indicate the existence of a ratio of something like 6 to 1 between recovery period and chronaxie over a wide temperature range.

The problem becomes still more suggestive in the light of the recently published data of Amberson (1930) on the effect of cooling upon the duration of the *absolute* refractory period. His results are represented by the third set of points in the figure. To make these values fit the general curve the ordinates must be plotted as 4 times the chronaxie at any temperature. Considering that the results of Amberson were obtained with a different

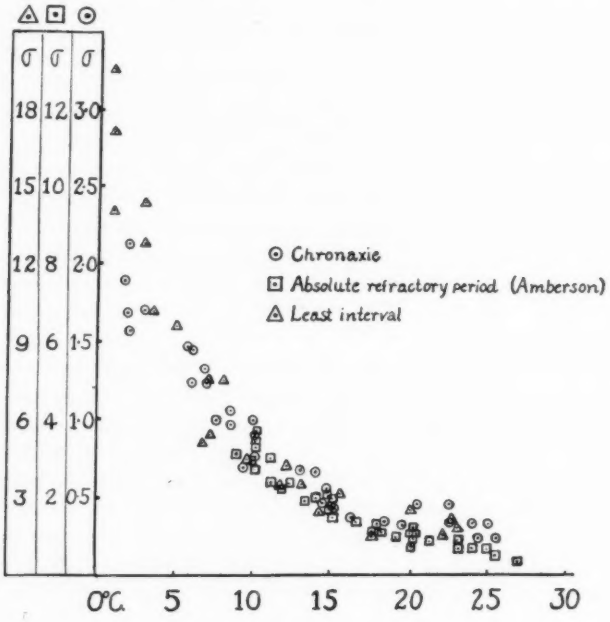

Fig. 1

procedure and with different frogs, the agreement with our results is surprisingly good. Amberson fits his data to an exponential curve, and calculates a temperature coefficient (Q_{10}) of 3.06 throughout a temperature range between 27°C. and 9°C. To the extent that our results coincide, it appears that chronaxie as well as the least interval have a similar Q_{10}.

Below 9°C. Amberson deduces the following values for the absolute refractory period: at 3°C., 6.8σ; at 4°C., 6.0σ; at 5°C., 5.4σ; at 8°C., 3.86σ. In the accompanying table are arranged values calculated from his curve for the refractory period at other temperatures; from these values are derived the theoretical chronaxies (assuming the latter to be

$\frac{1}{4}$ of the absolute refractory period). In the last column are given the values obtained in our experiments:

TEMPERATURE	ABSOLUTE REFRACTORY PERIOD—CALCULATED	CHRONAXIE—CALCULATED	CHRONAXIE FOUND
°C.	σ	σ	σ
3	6.8	1.7	1.7
6	4.8	1.2	1.46
7	4.3	1.07	1.24
8.5	3.6	0.9	0.96
			1.07

The significance of these figures—the approximate ratio of 6:4:1 between "total" refractory period, absolute refractory period, and chronaxie—remains to be determined. They all, of course, refer to certain aspects of nerve excitability in terms of time. Whether the figures imply a real functional relationship, or are simply an accidental numerical relation, we are unable at present to state.

SUMMARY

The effect of cooling the nerve upon least interval for muscular summation and duration of chronaxie has been studied in the sciatic-gastrocnemius preparation of R. pipiens. Both processes are prolonged in a fairly uniform manner; a ratio of 6 to 1 appears to hold at any given temperature. Making use of the data of Amberson, a ratio of 4 to 1 is pointed out between absolute refractory period and chronaxie. The significance of these relationships is not discussed.

We wish to express our gratitude to Dr. Hallowell Davis for his helpful advice and criticism in the course of this research.

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THE CONFLICT BETWEEN EXCITATORY AND INHIBITORY EFFECTS IN A SPINAL CENTER

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Received for publication June 23, 1930

Sherrington (1908b) described what he termed "algebraic summation" of excitatory and inhibitory effects in a reflex center. He had previously established that the normal reflex effects of stimulating an afferent nerve in a hind limb of a spinal or decerebrate animal (cat) were flexion of the leg on the stimulated side and extension of the opposite leg. The flexion reflex consists not merely in contraction of the flexor muscles, but also in relaxation of the extensor muscles through reflex inhibition; correspondingly the crossed extension reflex consists in contraction of extensors and inhibition of flexors. When two nerves on opposite sides of the animal were stimulated simultaneously, it was possible, by appropriate gradation of the strength of stimulation on the two sides, to attain a balancing or "algebraic summation" of the opposing effects. The muscle exhibited a partial contraction intermediate between that evoked by the unopposed excitatory stimulus and the relaxed state resulting from the inhibitory stimulus alone.

This balancing was mentioned by Adrian (1918) as a difficulty in the way of Lucas's (1911, 1917) proposed interpretation of reflex inhibition as depending on a phenomenon similar to the Wedensky effect (1885). Lucas had shown that the Wedensky effect, superficially resembling inhibition, depended on the conducting path being kept occupied by a series of impulses at such high frequency that each occurred in the relative refractory period following its predecessor and was therefore of subnormal size inadequate to excite the muscle. A nerve fiber so occupied was unable to give a full-sized response, and therefore constituted a total block which prevented excitation of the muscle through its motor nerve. Lucas's proposal to interpret reflex inhibition on this principle was part of his broader suggestion that before we conclude that the nerve centers use "some different process from that which is the basis of conduction in peripheral nerves", we should inquire with all care whether the peripheral-nerve type of conduction can give a basis for understanding central phenomena. The balancing of excitation and inhibition in the center, described by Sherrington, appears at first sight incompatible with Lucas's hypothesis. As Adrian pointed out, "the frequency of the impulses in the central paths should be,

if anything, greater when both nerves are stimulated than when the inhibitory nerve is acting by itself, and therefore the impulses should be still further reduced in intensity."

This difficulty has been met by relegating algebraic summation to the center as a whole, rather than to the individual motor neurone. It was observed that the effect only occurred when the inhibitory (flexor) stimuli were weak and therefore presumably left much of the extensor center uninhibited (Forbes, 1921, p. 288; 1922, p. 391). Central inhibition may perhaps be effected by a mechanism which establishes a total block in the approach to the individual motor neurone involved, and yet an apparent grading of activity in the center as a whole, through algebraic summation, may result from the total inhibition of some neurones while others are left free to respond.

Sherrington (1925; cf. Samojloff, A. and N. Kisseleff, 1927) has offered an interpretation of reflex function which postulates a central excitatory state (c.e.s.), differing from the peripheral nerve impulse in that it has no absolute refractory phase, and that it is capable of extensive gradations of intensity through the cumulative effect of summation. This hypothesis is supplemented by the assumption of an opposite state (c.i.s.) underlying inhibitory effects, which has the property of neutralizing the excitatory state. Gradations in the amount of reflex activity and the balancing of excitatory and inhibitory effects are simply treated as depending on the intensities of the excitatory and inhibitory states in the center. Fulton (1926) has designated this hypothesis the "chemical" theory of excitation and inhibition, for the reason that it is easy to visualize it in terms of chemical substances liberated in the central structures, but it should be noted that Sherrington remains non-committal as to the physical nature of the state. In support of this hypothesis Sherrington (1925) cited his previous observation (1909) that a constant inhibitory stimulus may produce marked relaxation when pitted against sub-maximal excitatory stimuli, but less relaxation of more powerful reflex contractions. This same observation was cited by Creed and Eccles (1928) as evidence that algebraic summation of excitation and inhibition occurs in *individual motor neurones.* These authors proceeded to confirm the observations, using the isometric myograph for the better quantitative evaluation of their reflex responses.

It should be noted that this observation of Sherrington, the similar observation of Graham Brown (1927), and the observations of Creed and Eccles, all showing decreased effect of a given inhibitory stimulus as the excitatory background increased in strength, and all cited by Creed and Eccles as evidence of the balancing of excitatory and inhibitory effects in the individual motor neurone, were all made on the flexor motor center, inhibited by the contralateral nerve, this inhibiton being part of the crossed extension reflex. As far as we are aware, no instance of this relation has been reported in the

case of ipsilateral inhibition of the extensor center. There are important differences between the flexor and extensor centers which render it unsafe to generalize about the central conflict between reflex excitation and inhibition, on the basis of observations made either on the flexor or extensor center alone. Probably the most important difference, in the present discussion, lies in the dominance of the flexion reflex. This is clearly shown by Sherrington's observation (1913) that when two afferent nerves from opposite hind limbs are simultaneously stimulated, the flexor muscles of both legs contract, and the extensor muscles of both legs relax. In other words, the flexion reflex completely dominates the crossed extension reflex on both sides. (Cf. Sherrington, 1908b.)

The case for dominance of the flexion reflex rests largely on these early observations by Sherrington. It was reinforced by a comparison of two series of experiments with similar apparatus. In this comparison the strength of stimulus required to inhibit the crossed extension reflex (Forbes, 1912a) was correlated with the action currents in the afferent nerve trunk (Forbes and Gregg, 1915; Forbes, 1921, pp. 286–289). So important a point should depend on more direct evidence. This fact, together with the fact that we know of no systematic study of the balancing of excitatory and inhibitory effects in the extensor center, as observed with the isometric myograph, comparable with that recorded by Creed and Eccles in the case of the flexor center, led us to perform the experiments described herein.

It is also noteworthy that Creed and Eccles cite observations of Sherrington in which "the same strength of stimulus of the inhibitory afferent evokes more relaxation when pitted against a larger reflex contraction than against a smaller one." This is the exact opposite of the relation described above, yet in the discussion it does not appear what conditions determine which type of result is found. Creed and Eccles show both types of result in their observations on the flexor center, in different parts of the range of excitatory stimuli. It is of interest to know whether a similar relation can be found in the extensor center.

In our experiments the knee extensor, vasto-crureus, was used as the indicator of reflex effect. It was connected with an isometric lever, in order to obtain the best possible quantitative measure of the motor-neurone discharge. To control the question whether the afferent nerve was maximally stimulated or not, it was deemed important to record its action currents at the same time that the reflex was being evoked. Just what constitutes a maximal stimulus is not altogether clear. Forbes and Gregg (1915) found that even after the limiting maximal value of the action current of the afferent nerve was attained, further increase in the strength of stimulus produced considerably greater central effects. Adrian and Forbes (1922) subsequently showed that when an inductorium with an iron core was used, some of this increase was traceable to repetitive excitation by a single

stimulus. By using apparatus which does not cause repetitive response to each stimulus, it is possible to eliminate that source of confusion. More recently, Erlanger and Gasser (1930) have shown that a nerve trunk contains fibers whose reflex effect is considerable, but whose threshold is relatively very high, and whose activity contributes little to the observed action potential. If, in spite of these fibers, complete inhibition is obtained while the action currents are demonstrably submaximal, it is clear that less than all the fibers of the afferent nerve have sufficed for total inhibition. It is also important to know that the response of the afferent nerve has remained constant throughout a given series of observations. For these reasons the action current control is an important feature of these experiments.

Apparatus. The string galvanometer and the recording camera employed in this research are the same as those previously used in this laboratory, but the whole outfit has been rebuilt and equipped with an isometric recording lever, arranged to give simultaneous mechanical and electrical records. The support on which the galvanometer and its source of light stand is a concrete pillar built up from its own foundations independent of the flooring. Above the floor level the pillar is shaped like a table, to provide accommodation in it beneath the galvanometer for the electrical amplifier described previously by Forbes and Thacher (1920). On top of the pillar is placed a layer of felt about half an inch in thickness, and on this rests a concrete slab 6 feet long, 4 feet wide, and 4 inches thick, supporting the galvanometer. Tests show that this support is almost completely free from vibration.

The source of light is a tungsten ribbon incandescent lamp, which takes an 18-ampere current at 6 volts. This source of light is found to be much steadier than a carbon arc, and to give sufficiently brilliant illumination for good photography at a magnification of 500 and at film speeds up to 30 cm. per second. The lamp is supplied from the 110 volt mains with a rheostat placed in series.

Musuclar contractions are recorded on the same film with the electrical record by an isometric myograph of the shadow type. The myograph is of the same general design as that described by Fulton (1926), but of very heavy construction. The torsion bar is a piece of tool steel, 6×6 mm. in cross section and 10 cm. long. One end is clamped rigidly, and the other end is filed down to a small rod-like projection which rests in a shallow notch in a horizontal supporting member. The bearing where the end of the torsion bar rests in its notch is thoroughly lubricated. A horizontal arm attached to the torsion bar is provided with a series of notches in which a hardened steel hook attached to the tendon may be placed. An aluminum recording arm 57.5 mm. long, braced diagonally to the end of the horizontal arm, extends upward through a hole in the tube of the galvano-

meter just behind the projection ocular. The upper part of the recording arm carries a pair of vertical hairs stretched between two horizontal jaws. For this we have used pieces of quartz fibers, such as are employed for the galvanometer string, with considerable success, and also threads of glue, drawn out thin and allowed to harden. Two recording hairs are employed, mounted parallel at such a distance from one another that when the first is about to leave the recording surface of the film the second will appear upon it. This arrangement virtually doubles the useful width of the recording film. With the adjustable ocular set in the usual position the movement of the recording hair is magnified 16 times. This gives a magnification of the tendon movement of from 28 to 120, depending on the notch in which the tendon hook is placed. When the longest lever arm is employed, as in this research, one kilogram of tension gives 2.70 mm. excursion on the film, and the excursion is proportional to tension over the entire range available. The natural period of this myograph when unattached to any muscle is 1500 per second. With this lever we have never been able to record an angle such as Fulton (1926) has described, although before we were aware that the angle was due to friction in the bearing (Cooper and Eccles, 1930) we made many efforts to obtain one by improving the rigidity of our fixation. All of our records show a typical rounded peak and smooth curve of relaxation.

The myograph, and also the supports for fixation, are carried on a concrete pillar, built up from the foundation independent of the pillar which supports the galvanometer. In this concrete pillar is set a steel post 7.6 cm. in diameter, carrying two movable iron collars, each provided with a horizontal arm cast in one piece with the collar. One of these carries the supporting rods for fixation of the animal, and the other the myograph. The vertical bearing surface of the myograph collar upon the post is 7.4 cm. The distance from the post to the optical axis of the recording system is 17.5 cm. Since the horizontal cast iron arm is 4.2 cm. in thickness, extreme rigidity is assured. The collar is free to turn about the post, and also to move vertically upon it, but it can be fixed in any position by two set screws. Fine vertical adjustment is obtained by means of a screw control. The horizontal arm is grooved on its upper surface, to carry a steel plate, 1.0 cm. thick, to which is clamped the base of the myograph. This plate can be moved along the axis of the horizontal arm by a screw, and clamped in any desired position. This affords the lateral adjustment of the myograph. It was found necessary to insert sheets of hard rubber between the steel plate and the myograph, in order to provide electrical insulation between the myograph and the supporting pillar. Unless this was done stray currents of considerable magnitude found their way into the galvanometer. We have measured potentials of several hundred millivolts between the steel support and the ground.

The rods which support the bony origin of the muscle pass up through holes in a board on which the cat lies. This board rests on a small table constructed independently of the supporting piliar. The legs of the animal board are of hard rubber to avoid accidental grounding of the animal other than by the recording electrodes, and are adjustable in height, to enable it to follow the movements of the support.

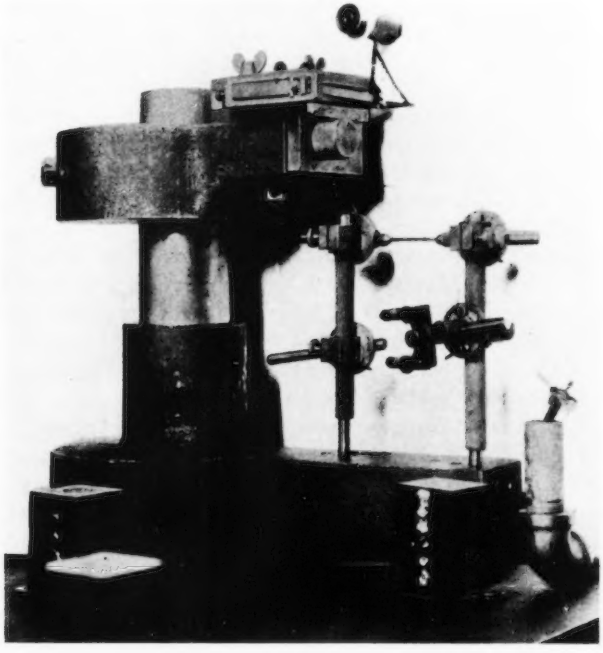

Fig. 1. Myograph and supports for fixation. The galvanometer is concealed behind a white screen to render the mechanical arrangements more clearly visible. The ocular is seen projecting through a hole in the screen, and the myograph lever is just below it. On the supports beneath the lever are mounted a drill and a femur clamp such as are used for fixation. At the left is the steel post with the two iron collars which carry the myograph and supporting rods. At the right are the galvanometer lead wires protruding from the iron pipe which serves as an electromagnetic shield.

In these experiments fixation of the limb was obtained by means of a drill passing through the lower end of the femur, and clamped at each end to one of the vertical supports. A double-jawed femur clamp was also applied on the medial aspect to the shaft of the femur, and was clamped in

its turn to one of the vertical supports. The clamps, like the myograph, were electrically insulated from their supports. This combination gave satisfactory mechanical rigidity for recording the contractions of the vasto-crureus muscle. The patellar tendon was cut between patella and tibia, and a hole drilled through the center of the patella to receive the lower end of the steel hook which passes over the arm of the myograph lever.

The particular advantage of this type of mechanical fixation lies in its solidity and compactness. Cat and myograph are firmly fixed with reference to one another on the same vertical support, and the whole is mounted on a pillar which is independent of vibrations of the building.

The recording camera was described by Forbes and Thacher (1920). It is mounted on an independent table at a distance of 102 cm. measured from the string of the galvanometer to the film. The revolving mirrors described by Forbes and Mann (1924) are mounted on a track in front of the camera, so that they can be drawn into position at a moment's notice, for examination of action currents.

A modification of our usual stimulating electrodes was designed with a particular view to avoiding traction on the nerve or accidental pinching during mechanical adjustment of the animal. A tube of hard rubber, 6.5 mm. outside diameter, 3.5 mm. inside diameter, and 25.0 mm. long, is provided with two silver wires, passing through it nearly tangentially. Outside the tube each is soldered to a very fine enamel insulated wire, and all exposed metallic portions are covered with a layer of "Ambroid", which serves as a flexible and waterproof insulating and supporting cement. The nerve is drawn through the tube, whose size is chosen to give a free fit though still insuring contact. The muscles are then stitched together loosely over the tube, and the animal can now be moved about freely, without fear of traction from the very flexible wires. We find these electrodes mechanically superior to the glass shield type suggested by Sherrington, and the nerve is thoroughly protected from drying and temperature changes. Very little difficulty has been experienced from shift of contact or from asphyxia during prolonged experiments.

METHOD. The animals (cats) were decerebrated under deep ether anesthesia by the guillotine method, transection being through the anterior colliculi. The crossed extension reflex was evoked by stimuli applied to the entire left sciatic nerve. It was recorded by contraction of the right vasto-crureus muscle. Inhibitory stimuli were applied to the right sciatic nerve. In the first experiments the entire right sciatic was employed, but in the last five experiments it was split into its component branches, peroneal and popliteal. The effects of stimulating these two branches proved to be so different that their separation rendered the picture less confusing. We therefore adopted the practice of making a complete set of observations with one branch alone, and then a complete set with the other.

All muscles in both legs which could have caused appreciable disturbing motion were paralyzed by section of their motor nerves. In one experiment we had left uncut one or two small twigs supplying the rectus femoris. This muscle, having a flexor component, caused a slight contractile effect during the application of the inhibitory stimuli. It proved a simple matter to discount this effect in analyzing the results.

The stimuli to the excitatory nerve were produced by a pair of coreless induction coils, connected in series, and balanced according to the method suggested by Bishop (cf. Bishop, Erlanger and Gasser, 1926), to minimize the electric artefact. The primary current was interrupted by means of a rotary interrupter, arranged to close the circuit fifty per cent of the time. This interrupter was tested by means of a string galvanometer several times during the research, to make sure that the makes and breaks were clean. The strength of the stimuli was regulated by varying the amount of resistance of the primary circuit. The inhibitory nerve was stimulated by means of the electron tube device described by Campbell (1929; cf. Forbes and Rice, 1929). Both stimulating devices were adjusted to give approximately 100 stimuli per second. In the case of the rotary interrupter this meant 50 interruptions per second, the make shocks as well as the breaks being effective in practically all observations.

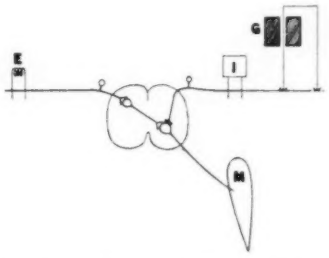

Fig. 2. Diagram of connections, showing schematic section of spinal cord. *E*, excitatory stimulating coil connected with left sciatic nerve. *I*, inhibitory stimulating apparatus applied to right afferent nerve (peroneal or popliteal). *G*, galvanometer for recording action currents. *M*, right vasto-crureus muscle.

In order to make quantitative records of the action currents in the inhibitory nerve, it was desired to lead them off monophasically. To this end it was necessary to place the leads at the peripheral cut end of the nerve, and place the stimulating electrodes central to the leads, close to the point where the nerve entered the moist chamber. This was placed as close as possible to the animal's leg, and the intervening space was well packed with moist cotton. The silver stimulating electrodes were 5.5 mm. apart. The recording electrodes, made of silver and coated with silver chloride, were 15 mm. apart at a distance of 18 mm. from the other pair.

Our standard procedure was to find the threshold of the inhibitory stimulus by means of the revolving mirrors; then to take a series of photographic records showing the size of action currents at all strengths of inhibitory stimulus from the threshold to the maximum value afforded by the appa-

ratus. The latter was usually nearly double the strength required to produce a maximal action current. We then proceeded to take records in series, the strength of the inhibitory stimulation remaining constant through each series, and the excitatory stimulus being varied in successive tests from threshold to maximal and then back to threshold again. In each test the excitatory stimulation was applied first. After it had been in progress about two seconds (long enough to enable the contraction to reach an approximate plateau), the inhibitory stimulus was applied for about two seconds. The inhibitory stimulus was then withdrawn, and about a second later the excitatory stimulus was withdrawn. Thus the excitatory stimulus was continued throughout each observation, and the contractile tension was registered before, during, and after concurrent inhibition. The interval between the beginnings of successive tests in the course of a series was thirty seconds. After the completion of each series a record was made of the inhibitory stimulus alone. A different strength of inhibitory stimulus was then selected, and a new series of observations was made, again covering the range of excitatory stimuli from threshold to maximal. In one experiment, after completion of the reflex observations, records were made of the action currents set up in the left sciatic nerve by the induction coils used to evoke the reflex. These observations covered the entire range from the threshold of the nerve to the maximum strength employed in the experiment. In this way we were able to correlate the reflex effect with the threshold and maximal values of the stimuli, as judged by the action current of the nerve.

Results. Experiments were performed on eight animals. Of these the first two yielded practically no results, but served as rehearsals for developing the technique. The remaining six preparations gave significant results, all agreeing in support of our main conclusions. Individual differences between the preparations in minor details yielded incidental observations of interest, which will be mentioned later. In each experiment we made a large number of individual tests; usually more than 200, the tests being grouped into more than 20 series. Practically every observation was repeated at least twice, in order to insure uniformity of behavior.

We have made a great number of measurements of the tension developed in the uninhibited reflex, and the level to which it fell under various strengths of inhibitory stimulation, but we hesitate to make more than roughly quantitative statements as to various types of behavior. As is already well known, the crossed extensor response does not appear until the strength of stimulus is raised well above the threshold for action currents in the afferent nerve, and the reflex continues to increase for stimulation strengths well above those apparently maximal for the action currents. Figure 3 illustrates this relationship, and we have already discussed its significance. In many cases the relation between response and stimulus is closely repro-

ducible from one test to another, while in other preparations alterations in the state of the spinal centers, probably in the nature of synaptic fatigue, tend to depress the responses toward the end of a series. Sometimes a prolonged recruitment makes measurements decidedly arbitrary, as no definite plateau is reached in the time we allowed before applying the inhibitory stimulation.

In order to plot graphically the results of inhibition, it would be very desirable to have some measurement of the intensity of the central excitatory state for our independent variable. Two alternatives suggest themselves: the strength of the excitatory stimulus,[1] and the tension developed in the

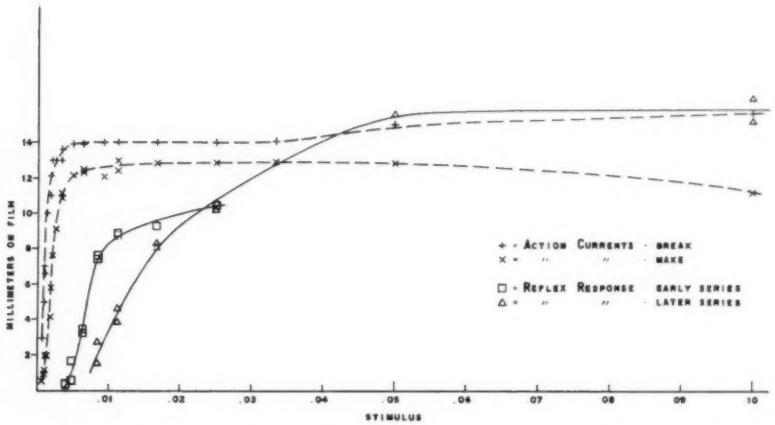

Fig. 3. Correlation of muscular tension in crossed extension reflex with strength of stimulus and with size of action currents in afferent nerve. January 15, 1930. Abscissae, strength of stimuli (reciprocal of resistance in primary circuit). Ordinates, millimeters excursion as measured on film. In the case of reflex response, 2.7 mm. = 1 kilogram.

excitatory plateau. Since the latter is an index of the number of neurones excited, it is obviously related to the central excitatory state, but in view of the all-or-none character of the motor neurone response, it cannot be regarded as a very simple function. Figure 4 illustrates its worst shortcoming. With a moderate strength of stimulation the excitatory plateau reaches, or very nearly reaches, a maximum value, but as the strength of excitatory stimulation is further increased the excitatory effect in the center becomes more intense, as is shown in the increased tension developed under simultaneous excitation and inhibition. But, on the other hand, if we plot

[1] For brevity the word stimulus is used to denote a continuous series of tetanizing stimuli, such as were regularly employed in this research.

against the strength of the excitatory stimulus, we have no guarantee that a given strength of stimulation always excites the same number of afferent fibers.

Our measurements are made more uncertain by the failure, in many cases, of excitatory and inhibitory balance to reach a definite level. Unless otherwise specified, we measure the degree of inhibition at the point of greatest relaxation, whether it is well maintained or not.

The observations were quite uniform in showing that maximal stimulation of the ipsilateral sciatic nerve causes complete inhibition of the extensor center, no matter how powerfully the opposite sciatic nerve is stimulated

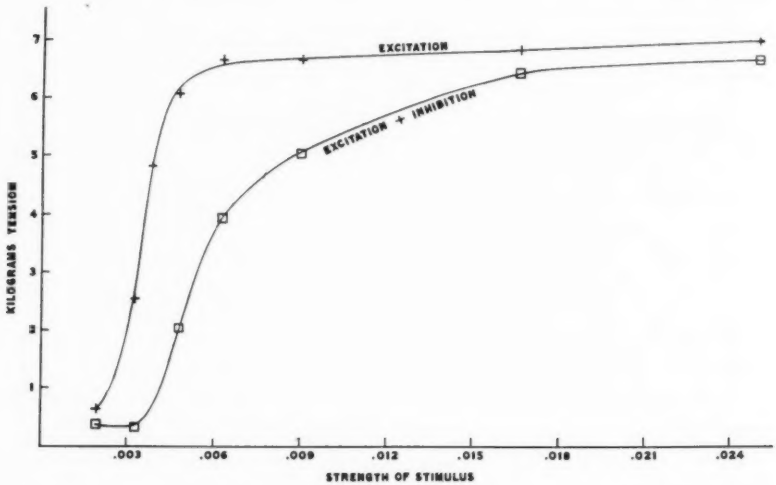

Fig. 4. Effect of varying the excitatory stimulus on the response to this stimulus alone and on the response to combined stimuli, with inhibitory stimulus of constant strength. November 14, 1929. Inhibitory stimuli of about one-half maximal value throughout series, applied to popliteal nerve.

at the same time. A single exception to this rule was observed, in which inhibitory stimuli slightly stronger than the apparent maximal value, as judged by the action currents in the afferent nerve, caused almost but not quite complete inhibition of an extremely strong crossed extension reflex, evoked by stimuli about fifty times the threshold strength for the excitatory nerve. This observation may be explained by the fact that the inhibitory stimuli, although apparently supramaximal, were probably still below the threshold of the fibers described by Gasser and Erlanger. Our strongest stimulus was less than double the apparent maximal value; had stronger stimuli been used, we are led to believe by the uniformity of our subsequent

observations that complete inhibition of even the strongest crossed extension reflex would have resulted.

In the last five experiments, we stimulated separately the two main component branches, peroneal and popliteal, of the ipsilateral sciatic nerve. In the first two of these experiments we also stimulated both branches simultaneously. It was evident that nothing was to be gained by so doing, and thereafter all our observations were made with stimulation of either the peroneal or the popliteal nerve alone, each nerve furnishing several series of tests in each experiment. One effect of splitting the nerve was to increase the effective strength of stimulus available with our apparatus. We found that to excite the peroneal branch required only from 50 per cent to 70 per cent of the E. M. F. necessary for the whole sciatic nerve. The observations with the separate branches of the nerve showed even more strikingly than those on the whole sciatic the dominance of the ipsilateral stimulus in the central effect. Maximal stimulation of either peroneal or popliteal nerve invariably sufficed to inhibit completely even the strongest crossed extension reflexes. The only apparent exception to this rule occurred in a case in which the inhibitory nerve was demonstrably damaged between the stimulating electrodes and the center. While peroneal or popliteal individually will inhibit the strongest crossed extension reflex, it is worth noting that stimulation of the whole sciatic gives a still greater inhibitory effect, as judged by the longer latency for the reappearance of tension after the end of inhibitory stimulation. Sometimes the ipsilateral stimuli had to be increased to a point just above what appeared to give maximal action currents, before the inhibition was complete against the maximum excitatory effect. In view of what has been said about the strong reflex effect of those fibers with high thresholds, whose response does not appreciably augment the action currents, we may safely conclude that when inhibition was not quite complete some afferent fibers remained unexcited. It is especially noteworthy that maximal stimulation of the peroneal nerve alone, which constitutes less than half of the sciatic nerve, sufficed to produce complete inhibition of the excitatory effect produced by the entire sciatic nerve on the other side.

Submaximal stimulation of the inhibitory nerve revealed many points of interest concerning the antagonism of reflex effects in the center. We found qualitative differences between the central effects of peroneal and popliteal stimulation. In general the central effect of stimulating the peroneal nerve was simpler and more purely inhibitory, and therefore will be described first. When the ipsilateral stimuli were nearly maximal, inhibition was almost always complete until the strongest excitatory reflexes were encountered. In some cases stimuli which were clearly submaximal sufficed to produce complete inhibition of even the strongest extensor reflex. When during concurrent stimulation the excitatory

effect appeared it would sometimes persist throughout the time of inhibitory stimulation, relaxation being partial but never complete. In other observations the onset of the inhibitory stimuli would be followed by complete inhibition, but after an interval of from 0.1 to 1 second the excitatory effect would break through and the muscle would contract slightly while the

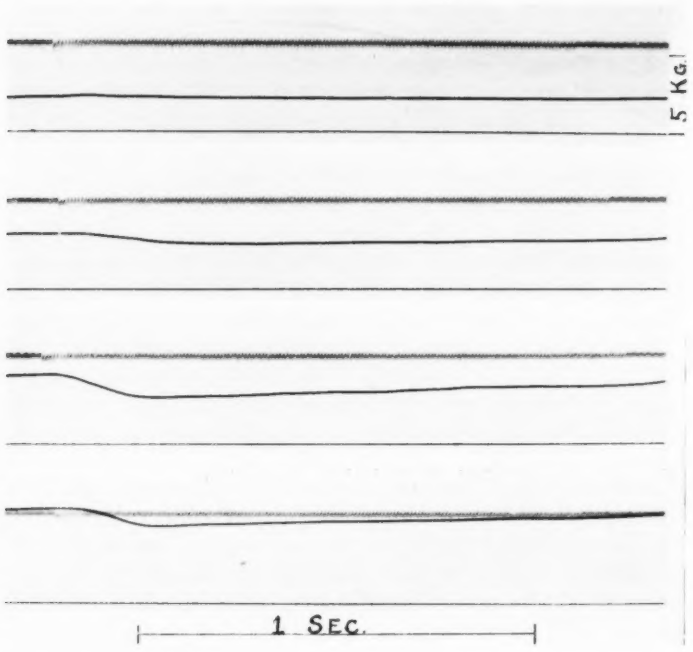

Fig. 5. Records of reflex contraction and action currents in inhibitory afferent nerve. Series with constant inhibitory stimulus (peroneal) and progressively increasing excitatory stimuli. November 26, 1929. Inhibitory stimulus, 1.3 (threshold, 1.0; maximal, 4, by action current). Excitatory stimuli were progressively stronger in successive records, reading from top to bottom. In all photographic records the top line records action currents, the second line contractile tension; below is the line of zero tension.

inhibitory stimuli were still being applied (fig. 13). As the inhibitory stimuli were made progressively weaker, smaller and smaller excitatory effects would suffice to break through the inhibition. With stimuli only slightly above threshold only partial inhibition of the reflex occurred at all strengths of the excitatory stimuli.

A significant fact is that with submaximal inhibitory stimuli the amount

of relaxation reached a maximum as the strength of reflex excitation was progressively increased, and then when the reflex response became very powerful, decreased again (figs. 5, 6, 7 and 8). This relation resembles that described by Creed and Eccles (1928) in the case of crossed inhibition of flexor contraction.

Observations on the popliteal nerve confirm those on the peroneal nerve in the most important features; that is, maximal stimulation regu-

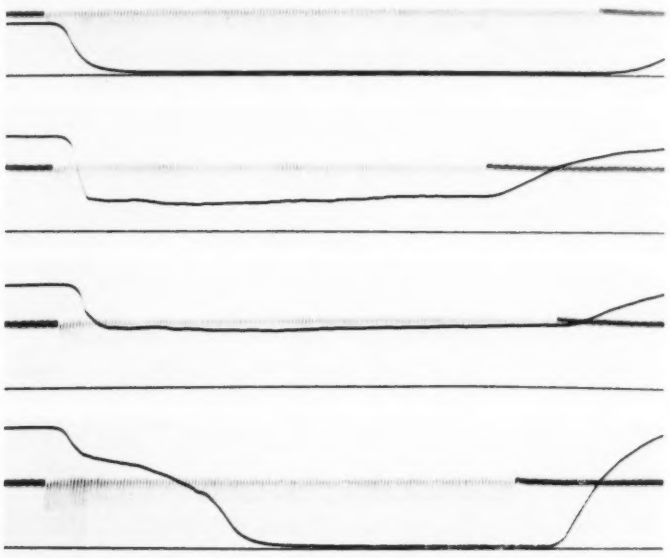

Fig. 6. Series similar to figure 5, but with stronger inhibitory stimulus (peroneal). Strength of stimulus 2.2 (threshold, 1.7; maximal, 4). Excitatory stimuli successively 0.00322, 0.00476, 0.00908, 0.02500, measured as reciprocal of primary resistance. November 14, 1929.

larly inhibited even the strongest of reflexes. Moderately submaximal stimuli showed essentially the same relation that was established in the case of peroneal nerve, inhibition being complete until excitation became strong, and then becoming less and less complete as the excitatory stimuli were made stronger and stronger. With weak stimuli, not much above threshold, applied to the popliteal nerve, a very different effect appeared. Sherrington and Sowton (1911a) showed that with weak stimuli the usual inhibitory effect on the ipsilateral extensor center was replaced by an

excitatory effect. This effect was tentatively ascribed by them to the presence of a different type of afferent fibers, with excitatory instead of inhibitory central connections. They noted that this effect was much more easily produced with the popliteal than with the peroneal nerve. Our observations are in striking agreement with theirs on this point, for in two out of the five preparations in which we separated the two nerves, the excitatory effect was clearly and regularly revealed on weak stimulation of the popliteal nerve, whereas in none of our experiments did it appear

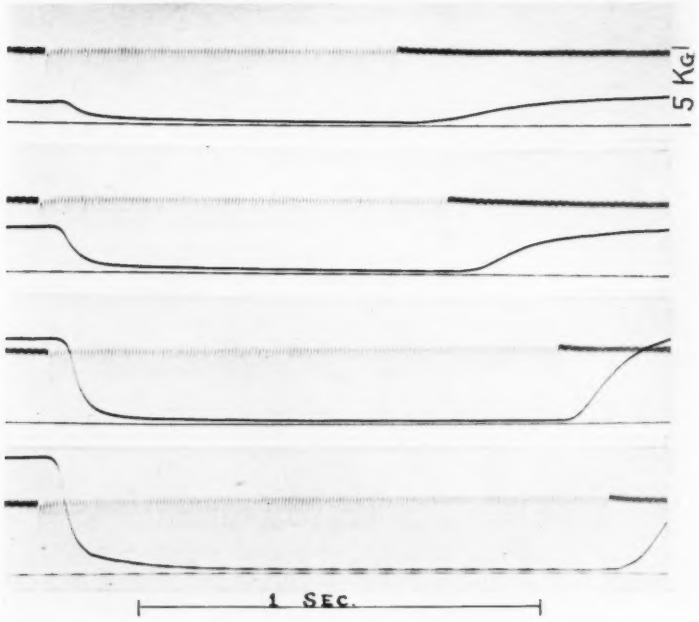

Fig. 7. Same preparation as figure 6. Still stronger inhibitory stimulus (strength, 3). Excitatory stimuli 0.00244, 0.00322, 0.00476, 0.01665.

in stimulation of the peroneal. A further observation of especial interest occurred in this connection. In a typical series, with a constant strength of ipsilateral stimuli, the effect would be inhibitory as long as the contralateral stimuli were weak. As the contralateral stimuli became stronger, and their excitatory effect on the center became greater, the effect of the constant ipsilateral stimuli changed from inhibitory to excitatory (see figs. 9 and 10). The important point here is that the effect of a given ipsilateral stimulus is changed from inhibitory to excitatory by changing the reflex background induced by contralateral stimulation.

These experiments gave opportunity for incidental study of post-inhibitory rebound. This increase in tension following the cessation of an inhibitory stimulus was seen in nearly every preparation under some conditions, and in one of them in almost every test. It varied from one preparation to another quite as much as the degree of resting tonus or the rate of recruitment of the crossed extension reflex. We were unable to correlate it with stimulation of the popliteal nerve, rather than the peroneal, or with the presence of an excitatory component in the response to stimulation in the inhibitory nerve. Sometimes an excitatory effect may result

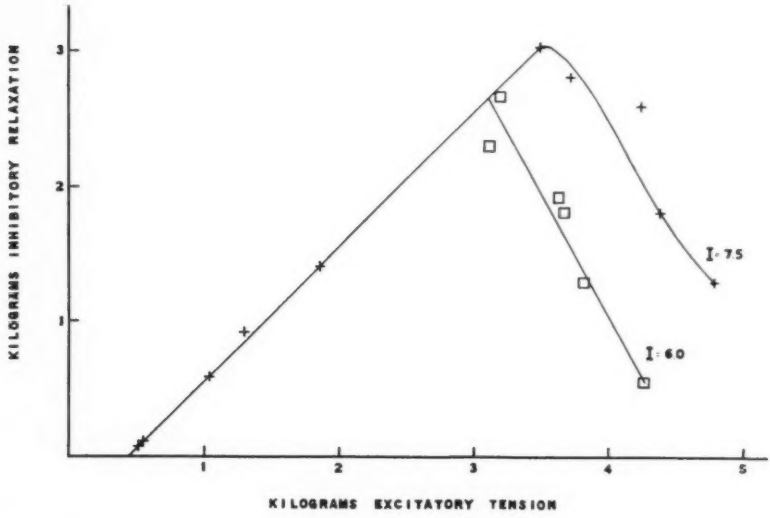

Fig. 8. Graphs of inhibitory relaxation from two series of records similar to those of figures 5–7, peroneal nerve. January 15, 1930. Abscissae, tension under excitatory stimulus alone; ordinates, amount of relaxation under concurrent inhibition. One graph shows series with inhibitory stimulus of strength 6, the other 7.5; threshold 2.5.

from a weak inhibitory stimulus applied against a strong excitatory background, and yet no rebound follow the cessation of inhibition. Yet in spite of the individual differences between preparations, the rebound almost invariably appeared following a very strong ipsilateral stimulus applied without an excitatory background. The rebound became larger and appeared with shorter latency as the strength of stimulus was increased, and the action currents from the afferent nerve showed a small but definite concomitant increase. The rebound is definitely associated with sensory fibers of relatively high threshold. As the strength of the inhibitory stimulus is increased from the threshold value, the first effect is to abolish the

resting tone of the extensor muscle. Knee jerks may still be uninhibited, and may seem increased rather than diminished. This is because the maximum tension reached remains unchanged, but the contraction starts from a lower base-line. Further increase of the inhibitory stimulus

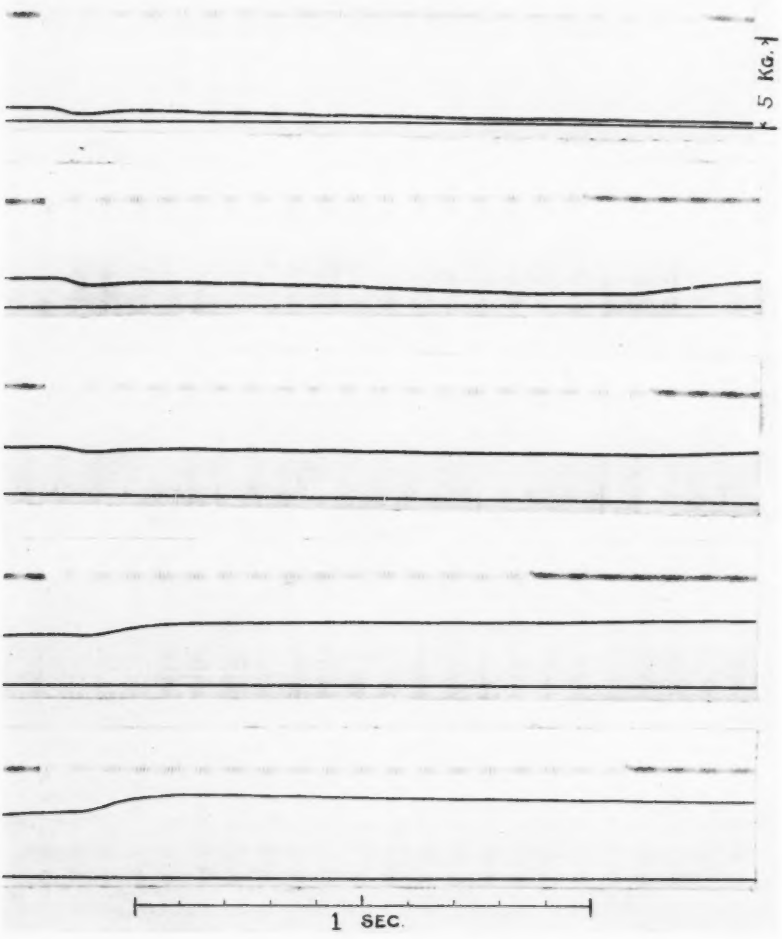

Fig. 9. Series of records showing change of central effect of weak ipsilateral stimulus (popliteal) from inhibition to excitation, as excitatory background is increased. November 8, 1929. Inhibitory stimulus, 2.5 (threshold, 1.8; maximal, 6). Excitatory stimuli, 0.00196, 0.00244, 0.00322, 0.00476, 0.00908.

diminishes and finally suppresses the knee jerks. As a rule rebound does not appear until a strength of stimulus has been reached which almost if not entirely suppresses the knee jerks.

The preparations in which the crossed extension reflex recruited slowly offer a clue to the interpretation of the rebound phenomenon. Very often the tension developed following application of the inhibitory stimulation is much greater than had been attained during the previous excitatory stimulation. It appears from inspection of the records (cf. fig. 11) that

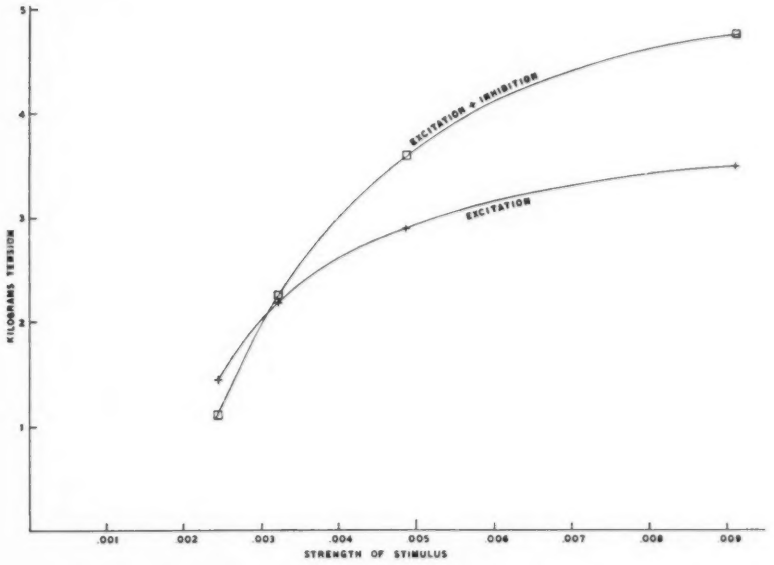

Fig. 10. Graph showing the same effect illustrated in figure 9, and drawn from part of the same series of observations. November 8, 1929. Inhibitory stimulus, 2.5 throughout. Abscissae, strength of excitatory stimuli. Ordinates, contractile tension.

the increase represents merely a continuation of the process of recruitment, which has continued at an uninterrupted pace although masked by the inhibition. This suggests that the recruitment and the inhibition take place in different parts of the central mechanism, and that the inhibition is closer to the final motor neurone than is the locus of recruitment (cf. Liddell and Sherrington, 1923). This seems to indicate some factor underlying recruitment, other than progressive release from "autogenous" inhibition (Fulton and Liddell, 1925). All of this suggests that post-inhibitory rebound is primarily the manifestation of a masked excitatory

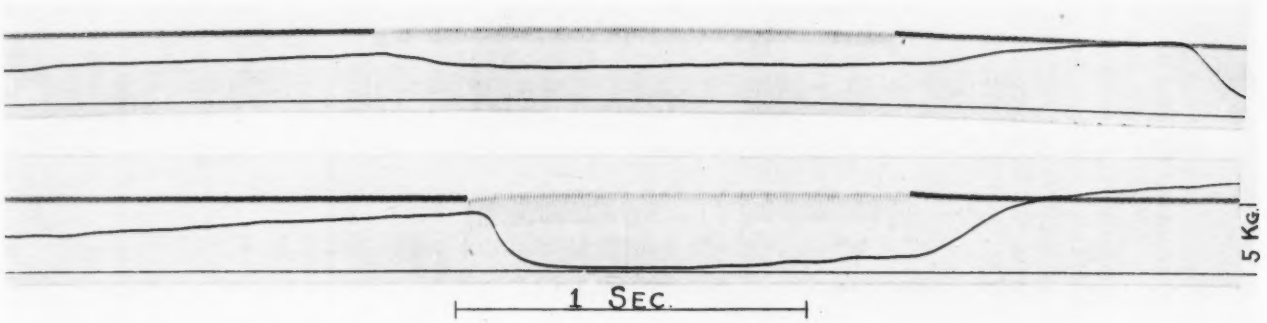

Fig. 11. Records showing post-inhibitory rebound, in which tension continues to increase at the same rate as before inhibition. Popliteal nerve. November 19, 1929. Upper record; excitatory stim., 0.00625; inhibitory stim., 3. Lower record; excit. stim., 0.00908; inhib. stim., 5. Inhibitory threshold 2.6; max. 6.

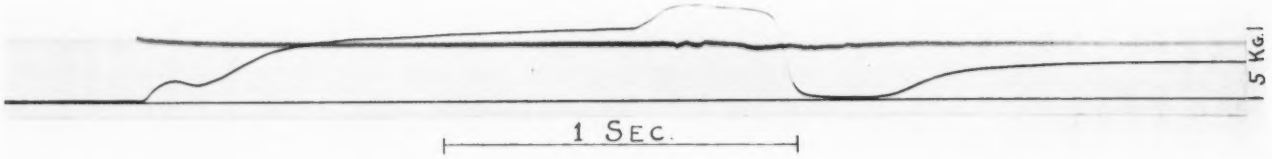

Fig. 12. Record of complex sequence of contraction and relaxation following cessation of afferent stimuli (see text). End of ipsilateral stimulus shown by cessation of action currents. End of contralateral stimulus (about the middle of record) marked by abrupt increase in tension. November 8, 1929. Excitatory stim., 0.0500. Inhibitory stim. (to whole sciatic), 6. (Threshold, 3; max., 8.)

component in the central effect. As a rule the afferent fibers which elicit these excitatory components seem to have higher thresholds than the inhibitory, and we obtain evidence of their existence primarily from these rebound phenomena.

Another phenomenon fundamentally similar in character, which we occasionally observed following very strong excitatory stimulation, was an abrupt although transient increase in tension immediately following the end of excitatory stimulation. This appeared with great regularity in all but one preparation, and usually at about the same strength of stimulation in all. The obvious explanation is that the excitatory nerve contains an inhibitory component of high threshold (cf. Eccles and Granit, 1929, p. 113), which inhibits groups of motor neurones which would otherwise be called into action by the increasing stimulus. This post-excitatory contraction appears at approximately the same strength of stimulation as vigorous activity in the fore limbs. Both the excitatory and inhibitory fibers controlling these neurones have a high threshold. The excitatory effect in the nerve centers outlasts the inhibitory, and appears as a terminal excitatory effect. A similar effect under light etherization was described by Forbes (1921) and adduced as evidence for a dual central influence of afferent stimulation. Occasionally a more complicated series of events ensued after cessation of all afferent stimulation, as follows: post-excitatory increase of tension, rapid fall of tension to zero, characteristic of central inhibitory effects, and finally a typical post-inhibitory rebound (fig. 12).

Many tests yield evidence of synaptic fatigue. For example: excitation may break through an intercurrent inhibition after the latter has been complete for half a second or more (see fig. 13 A). This phenomenon probably depends on fatigue of the inhibitory process, although it might alternatively be described as a gradual accumulation of sufficient excitatory effect to break through a constant inhibition (fig. 11). Many of our records are not as convincing as that in figure 13 A, because in some instances, when excitation breaks through the inhibition, it can be correlated with gross irregularities in the action currents, possibly due to slight shift of contact on the stimulating electrodes. However, a slight falling off to a well maintained plateau due to "equilibration" is characteristic (see Forbes and Rice, 1929), and does not imply that the central effectiveness of the stimulation has been diminished, and in many cases the action current control appeared to indicate a steady state of afferent stimulation. A really steady balance between excitatory and inhibitory effects is the exception rather than the rule. Most of our measurements, from which our figures are plotted, are made of the minimum tension which was reached in the course of inhibition. This minimum may be in the form of a very flat curve, or it may be a decidedly sharp notch in the tension curve, depending on whether the excitation breaks through simultaneously in a

large number of motor neurones, or whether an approximately steady state is reached.

Occasionally the reverse effect to that described may be seen; that is, a gradual inhibition of an excitatory plateau. Figure 13 B illustrates this, and it is a point of particular interest that this record was taken only 30 seconds after figure 13 A. Conditions differed in that the second test was made with stronger contralateral excitatory stimulation, but the inhibitory stimulation was the same in both. With the weaker excitatory stimulation (fig. 13 A) inhibition prevailed more promptly, but was not maintained. With the stronger excitatory stimulation, inhibition was more gradual, but persisted. In this case it may be that certain inhibitory fibers in the

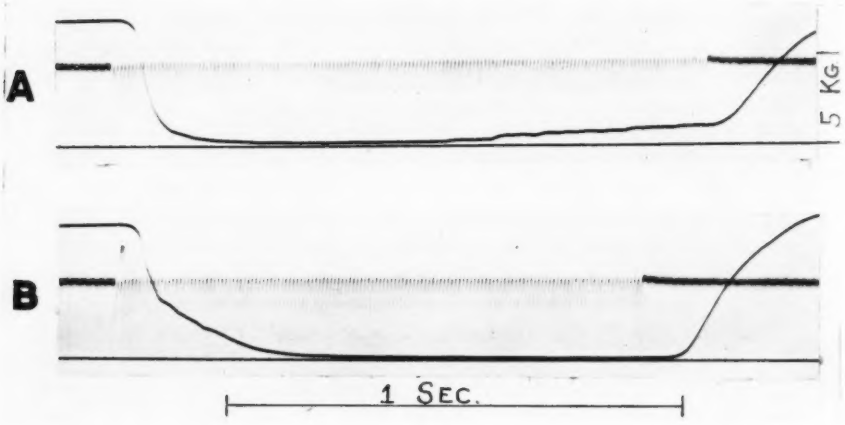

Fig. 13. Records showing evidence of central fatigue. November 14, 1929. A shows excitation gradually breaking through inhibition; B, excitation gradually yielding to inhibition. Excit. stim. in A, 0.00908; in B, 0.01665. Inhib. stim. (peroneal), 2.5 in both. (Threshold, 1.7; max., 4.)

contralateral nerve were excited and strengthened the inhibitory effect. That the difference was not due to a persistent central fatigue is shown by the fact that the effect recorded in figure 13 A was duplicated half a minute after figure 13 B was made.

In view of the pronounced activity caused by strong stimulation of either nerve, and in some cases the changes in tonus which may outlast the thirty second interval between tests, it is not surprising that we should find occasional differences in the response of the animal when a given observation is repeated. In fact, it is rather surprising that the results are as closely reproducible as figures 4 and 14 show them to be. Figure 14 represents a case in which seventy-five tests intervened between the

observations expressed by the first and second set of curves. The tensions under excitation and inhibition combined are nearly constant, although the response to excitation alone has fallen by thirty or forty per cent.

Occasionally a well marked rhythm, having no relation to the rhythm of

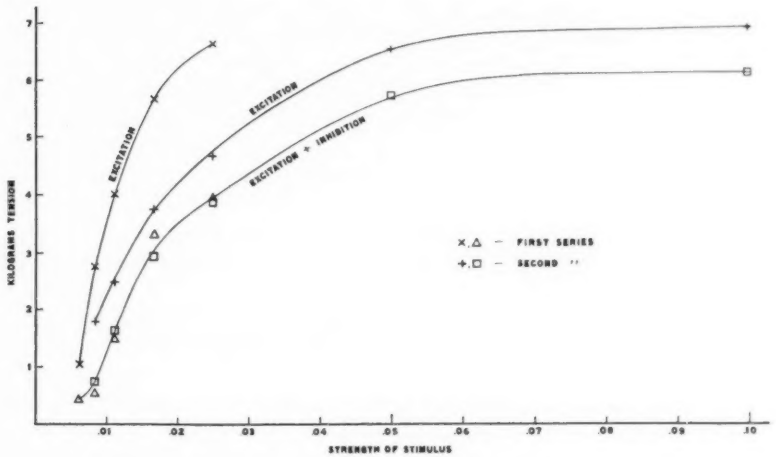

Fig. 14. Graph showing approximate constancy of balance between central excitation and inhibition, in two series of records made 50 minutes apart (see text). January 15, 1930. Abscissae, excitatory stimuli. Ordinates, contractile tension. Inhibitory stimuli (popliteal), 4 in both series (threshold, 2.5; max., 5).

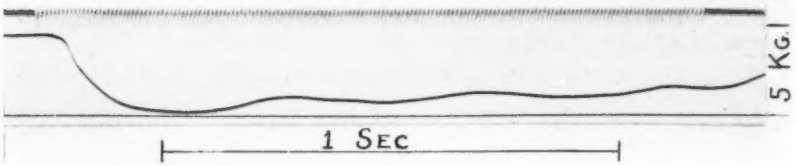

Fig. 15. Record showing rhythmic contractions during balance between central excitation and inhibition. November 26, 1929. Excit. stim., 0.00476. Inhib. stim. (peroneal), 1.7. (Threshold, 1.0; max., 5.)

afferent stimulation, appears in the myograph record during the balance between excitation and inhibition (cf. Graham Brown, 1912; Forbes, 1912b). These rhythms vary from perhaps 5 to 40 per second (fig. 15). When slow they show a smooth gradual ascent and descent like a progression reflex. When more rapid the jerky ascent and descent of the lever

shows that a large group of motor neurones is discharging intermittently, at a rate too slow to produce mechanical fusion. It is possible that the only difference between this and the usual discharge is that a large number of neurones have become synchronized in their activity. The more frequent occurrence of rhythm under inhibition recalls the observation by Dusser de Barenne and Brevée (1926) of a rhythmic discharge in the motor neurones when the spinal cord is painted with novocaine. In many instances, however, these apparent rhythms are purely fortuitous, as far as the nerve centers are concerned. The action currents recorded from the inhibitory nerve sometimes show a definite rhythmic modulation in size, and the myograph merely reflects the variation in intensity of inhibitory effect. At other times, the excitatory plateau may show a well marked rhythm in absence of inhibitory stimulation. Finally, we must remember the step-like character of recruitment. If these steps are more or less uniform and evenly spaced, they give an impression of a definite rhythm.

Discussion. Our observations have established the dominance of the flexion reflex in the sense that maximal stimulation of the ipsilateral afferent nerve completely inhibits the crossed extension reflex evoked by maximal stimulation of the contralateral nerve. They also show that in the extensor center, as well as in the flexor center studied by Creed and Eccles, inhibition by a constant submaximal stimulus increases with increase in excitatory background up to a maximum and then decreases. The chief difference between the two centers in this respect seems to be that in the flexor center the inhibitory stimulus may be ineffective against a weak excitation and yet suffice to inhibit a stronger excitatory effect, apparently due to the increasing overlap of the central fields of action. In the extensor center, on the contrary, if the ipsilateral stimulus is strong enough to inhibit a moderately strong excitatory effect, it will completely inhibit a weaker one. This difference seems to be an aspect of the dominance of the ipsilateral reflex.

Let us consider the bearing of these observations on the question whether we may interpret reflex function in terms of nerve impulses alike in kind to those in peripheral nerve, or whether we must postulate some qualitatively different activity in the central structures. Although various working hypotheses may be formulated, these two alternative views seem to be best represented by the hypotheses of Lucas and of Sherrington. That of Lucas takes the demonstrated summation of impulses in peripheral nerve as the prototype of reflex summation, and the Wedensky effect as the clue to reflex inhibition; that of Sherrington postulates excitatory and inhibitory states (c.e.s. and c.i.s.) in the center, graded in intensity, cumulative in effect, and not subject to refractory phase.

This latter hypothesis was put forward tentatively by Sherrington (1925) and vigorously endorsed by Fulton (1926). Creed and Eccles (1928) in

their discussion of inhibition appear to assume tacitly that this proposed concept of central excitation and inhibition is definitely established. Sherrington (1929, p. 343) intimates that the fact of central summation requires some such postulate.

Let us examine the available evidence and see whether any of it is crucial in forcing us to abandon as a working hypothesis either interpretation of reflex function. Sherrington's hypothesis is so flexible that it readily meets the requirements imposed by the observed facts of reflex behavior; indeed the apparently smooth gradations of excitatory and inhibitory effects in the center are suggestive of underlying states capable of the most delicate gradations, rather than of impulses of the all-or-none type. Yet it is in one sense simpler to interpret reflex function in terms of a single known type of response than to postulate a new type. Until the need of a new type is proved, its final acceptance is unwarranted. Lucas's hypothesis would offer a schema in which the all-or-none type of response is used as a single basis of analysis. The chief obstacles in the way of this interpretation seem to be (1), summation of central effects, and (2), the apparent possibility of graded balancing of the opposed excitatory and inhibitory effects in the individual motor neurone. As to the first,—we are not aware of any new features of reflex summation which are more difficult to interpret in terms of the Lucas hypothesis than those which were known when Adrian and Lucas first demonstrated summation of peripheral nerve impulses in 1912. They first established the fact that the nerve-muscle preparation, a conducting path responding throughout its length on the all-or-none principle, can nevertheless, under modifications induced by fatigue or narcosis, exhibit summation of successive impulses in a way that might furnish a possible basis for the cumulative effects so strikingly present in the reflex arc. Clearly, under certain conditions, the nerve impulse can create some persistent effect which outlasts the refractory phase and makes possible the summation of successive disturbances. Recently Levin (1927) has demonstrated a persistence of the electric response in a crustacean non-medullated nerve. On repeated stimulation, this "retention of action current" is cumulative through a considerable series of impulses. Amberson and Downing (1929) have shown the presence of a similar, though less marked, persistence of action potential in vertebrate medullated nerve. These form a possible basis for the summation of successive nerve impulses, conceivably applicable to the reflex arc. In short, the facts of summation do not force us to accept the postulate of a central excitatory state essentially different from the peripheral nerve impulse.

Let us now turn to the evidence in favor of the graded balancing, or algebraic summation, of excitatory and inhibitory effects in the individual motor neurones. The essence of the argument, as put forward by Creed

and Eccles, seems to be that a given inhibitory stimulus, sufficing to inhibit a certain number of motor neurones when these are excited by a moderate stimulus, inhibits fewer motor neurones when the excitatory stimulus is stronger. If reflex inhibition acted as a total block in the individual central path involved, and the apparent graded balancing in the center as a whole were due to gradation in the number of motor neurones inhibited, a given strength of inhibitory stimulus should block a fixed number of motor neurones, and no increase in excitatory effect should emancipate any of them from the block. The observed fact of increased excitation breaking through the effect of a constant inhibitory stimulus suggests, therefore, the grading of a central excitatory state in the *individual* motor neurone. We have pointed out that all the instances of this effect cited by Sherrington and by Creed and Eccles are those of crossed inhibition of flexors. Our experiments now show that a similar effect may occur in the extensor center, provided the inhibitory stimulus be submaximal. It is evident that inhibition which is apparently complete for moderate excitation, can be overwhelmed by strong excitation. The increased excitatory effect has not merely added more motor units to the number previously involved, but has robbed the inhibitory stimulus of the power to inhibit as many motor neurones as it did when acting against the weaker excitatory background.

It was shown in previous papers (Forbes, 1921, 1922) that a schema could be provided which would explain in terms of the Lucas hypothesis the dominance of the ipsilateral flexion reflex, and at the same time account for other facts of reciprocal innervation, notably the reciprocating rhythm of antagonists. Both Sherrington (1906) and Graham Brown (1914) have described conditions of rhythmic alternation of flexion and extension in absence of all proprioceptive impulses or other intermittent external sources of excitation. These rhythmic effects may be fundamentally akin to the oscillations occurring when excitatory and inhibitory effects are balanced. Instances of these have been described by Graham Brown (1912), by Forbes (1912), and in our present observations, and seem to emphasize the instability of the balance. Some mechanism analogous to the valves of a reciprocating steam engine may provide a tendency for each center to carry its activity beyond the point of balance and then undergo a reversal. The mechanism provided for this reciprocation in the above mentioned schema would suffice to explain the present observations.

The question now arises whether Sherrington's postulate of graded central excitatory and inhibitory states may not be so much simpler than the schema required by the Lucas hypothesis that nothing is to be gained by further speculation as to intricate arrangements of fibers. In this connection it should be noted that Sherrington's hypothesis, as hitherto

developed, makes no provision for these rhythmic phenomena. Central states acting like chemical substances would attain a steady equilibrium of balanced activity unless some periodic influence were brought to bear, to swing the balance first one way and then the other. Thus if we accept Sherrington's view we must also adopt some subsidiary hypothesis to explain the rhythmic shift of balance. The necessary condition might be found in fluctuations of threshold in the motor neurone, or in fatigue of the synapse through which it is approached.

Our observations have a bearing on the nature of rebound contraction. Sherrington has usually regarded this phenomenon as a "post-inhibitory exaltation" of the central excitatory state. The tendency to intrinsic rhythm, in which flexion and extension automatically succeed one another without the intervention of proprioceptive impulses, tends to support this view of rebound. The effect might be ascribed to the activation of an accessory mechanism in the center, as suggested above. On the other hand, the concept of post-inhibitory exaltation has carried with it the implication of the damming up of a stream of energy. It has already been noted (Forbes, 1912a) that the damming up of excitatory energy cannot account for some of the facts which Sherrington (1908a) has observed in connection with rebound. He found an optimum duration of the inhibitory stimulus which caused a maximum of rebound, and beyond which rebound became progressively less. If it were a simple result of a damming up of a stream of energy, the longer this stream was held in check, the greater should be the rebound on its release.

Sherrington and Sowton (1911b) proposed another interpretation of rebound, namely, that it was due to the presence in the afferent nerve of fibers with excitatory, as well as inhibitory central connections. On this view the inhibitory effect is dominant during the application of the stimulus, but the excitatory effect, having the longer after-discharge, asserts itself when the afferent stimulus is withdrawn. Forbes (1921) showed an effect under light etherization resembling rebound contraction, which seemed most easily interpreted on the same view of dual central effects. Other evidence, such as the reversal of central effects by chloroform, and by altering the character of the electrical stimuli (Sherrington and Sowton, 1911a), are most simply interpreted on a similar assumption that the afferent nerve contains some fibers with central connections causing flexion, and other fibers with central connections causing extension. McCouch and Alpers (1929) have furnished more detailed evidence in support of the same view.

Our observations lend some support to this view. In the first place, the rebound contraction appears to have a fairly definite threshold, suggesting that its appearance after inhibition depends on the peripheral stimuli being strong enough to excite a certain group of fibers with higher

thresholds than those causing inhibition alone. Again, the appearance of a post-excitatory contraction in most of our observations in which the excitatory stimuli exceeded a certain rather high value, strongly suggests the presence in the contralateral nerve of fibers causing inhibition of the extensor motor neurones, an effect directly opposite to the dominant crossed extension reflex. Since the extension reflex has a more prolonged after-discharge than the flexor effect, the cessation of contralateral stimulation is promptly followed by activity in those motor neurones which have been inhibited by the flexor component of the stimulus.

Altogether there seems to be a large body of evidence pointing toward the view that each afferent nerve contains antagonistic elements, and that this mixed effect is a clue to rebound. On the other hand, we cannot deny that rebound may be in part due also to the central mechanism which creates a tendency to rhythmic alternation of antagonistic reflex effects.

Interpretation of the spinal reflexes in terms of nerve impulses of the all-or-none type, according to the Lucas hypothesis, encounters other difficulties besides those mentioned above. In particular there are two observations mentioned by Sherrington (1929). One is the fact that a single inhibitory stimulus applied during the after-discharge of the extensor muscle causes an abrupt decline of contraction to a level which is then maintained without change, until such time as the after-discharge would have subsided to the same level of contraction, had it not been inhibited. This observation shows that the transient inhibition checks the discharge in just those motor units whose activity is most nearly ready to cease spontaneously. The other observation is that inhibition can be made to produce a graded slowing of the frequency of discharge of motor impulses in an individual motor neurone. Both of these observations strongly suggest the possibility of fine gradation in the excitatory and inhibitory states bearing on the individual motor neurone.

Let us see if these effects can in any way be explained in terms of the Lucas hypothesis. Figure 16 shows a schematic motor neurone, in the dendrite of which we may picture the conflict between the excitatory and inhibitory reflexes. The evidence of Sherrington (1929) and Lutz (1918) shows conclusively that a reflex effect almost invariably requires afferent impulses in more than one fiber of the sensory nerve, that is, it depends on central convergence of individual impulses. Let us assume, therefore, that to excite the individual motor neurone, impulses must converge and sum their effects at the junction of such branches of the dendrite as are shown at *E* in figure 16 A. Such a convergence will result in an effective disturbance penetrating to the cell body and setting up a motor impulse. Inhibition of the Wedensky type may be conditioned by a refractory period in part of the conducting path so brief that each impulse approaching it through the afferent fiber sets up in it a succession of impulses of high

frequency. In our schema this part of the path is denoted *I*. These high frequency impulses, on arrival at that part of the dendrite, *C*, which is common to the excitatory and inhibitory path, set up in it a series of impulses of such frequency that each is in the relative refractory period following its predecessor, and is therefore subnormal. Farther along the path is a region of relatively high threshold, which must be passed before the disturbance can reach the cell body. The subnormal impulses arriving from the inhibitory path not only fail to excite this region, but create a block, which obstructs impulses arriving through the excitatory path *E*.[2]

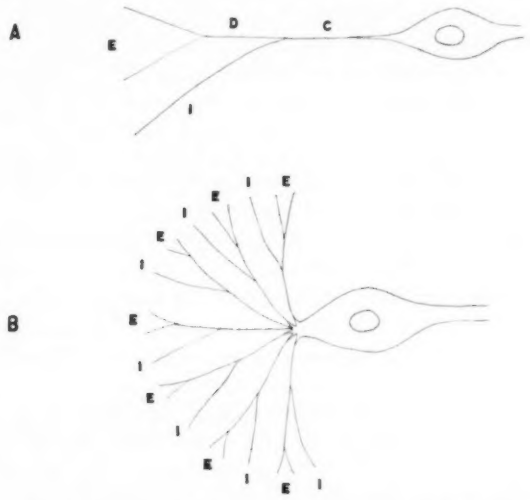

Fig. 16. Diagram of hypothetical dendrite (see text). A, arrangement of typical branches to provide a possible mechanism for inhibition. B, elaboration of schema to provide statistically for gradation of excitatory effect in individual motor neurone.

It is of cardinal importance to note that the frequency which we are postulating as a condition for central inhibition has nothing to do with the frequency of peripheral stimulation. This is obvious from the fact that a single shock applied to an afferent nerve will produce central inhibition. The only way in which a schema embodying the Wedensky type of inhibition can be made to square with the facts is to assume that the inhibitory rhythm of response is an inherent characteristic of the conducting path

[2] The schema illustrated in this figure is a development of that shown in a previous communication (Forbes, 1922, fig. 3), but it has been modified in the light of subsequent research (Davis, Forbes, Brunswick and Hopkins, 1926), which led to the abandonment of decremental conduction as an essential factor.

through which the inhibitory effects are produced. The schema must provide for central summation of inhibitory effects (see Sherrington, 1929, p. 355). This simply calls for the convergence of reinforcing inhibitory paths upstream from the point where the rhythm which is assumed to block the path becomes established.

In figure 16 B the schema is elaborated to account for the apparent gradation of excitatory and inhibitory effects impinging on the motor neurone. The picture is certainly no more complex than that which is directly revealed to us by histology. We may treat the branches of the dendrite statistically, and in discussions based on gradation in the central excitatory and inhibitory states we may substitute gradation in the number of inhibitory and excitatory branches effectively excited. Specifically, this schema will provide for the graded frequency of discharge of nerve impulses in the individual motor neurone under various grades of inhibitory stimulation, on the ground that the larger the number of inhibitory branches establishing blocks at their meeting points with the excitatory branches, the fewer excitatory branches will be free to bring in the disturbances which excite the axon of the motor neurone.

To this interpretation it may be objected that the crowding of impulses in the excitatory branches would tend to produce at their confluence that very effect—high frequency, and therefore subnormal impulses—which we are invoking to explain inhibition. An excess of excitation, therefore, should produce inhibition, instead of overpowering it. This difficulty may be answered by postulating that the refractory period of the part of the dendrite where the excitatory branches first meet shall be relatively long, so that only a low frequency can be transmitted through it, and that beyond it shall be a region of relatively low threshold, so that no amount of crowding in the smaller branches can set up an inhibitory frequency in the main common branch, *D*. This is the exact opposite of the condition postulated for the inhibitory branch, in which there is a region of short refractory period, followed by one of high threshold.

It is apparent that the process of summation at the point of junction of the dendrite branches differs little from that postulated by Sherrington as the central excitatory state. The activity of the central neurone is supposed to be a function of the excitation level maintained by a greater or smaller volume of impulses arriving at a junction point, and the frequency of discharge of the neurone is determined by its rate of recovery as related to this excitation level. The fundamental difference from Sherrington's hypothesis lies in relegating inhibition to the outlying portions of the dendrite where a greater or smaller proportion of the excitatory pathways are supposed to be blocked.

It cannot be too strongly emphasized that no schema which we are now in a position to set up is in the least degree probable. The apparently

far-fetched complexity of the schema here outlined is justified only as serving to show that no fact yet known has closed the door finally to a possible interpretation of reflexes in terms of such impulses as we know in peripheral nerves. It should be noted that Fulton's apparent refutation of the Lucas hypothesis, based on the long duration of after-discharge (1926, pp. 347–348), overlooks the possibility of central reverberation, or repeated traversing of the same conducting path.

We may conclude that, pending further evidence, the question whether we may interpret reflex function in terms of nerve impulses of the all-or-none type throughout the conducting path must remain open. We may yet find that instead of assuming the reflex center to have evolved a qualitatively different type of function from that found in peripheral nerve, we need only assume that the medullated nerve of vertebrates has evolved to its high degree of efficiency by minimizing fatigue and the associated properties found in more primitive types of peripheral nerve. Hill's suggestion (1929) that the primitive nerve of the crustacea, with its greater susceptibility to fatigue and to lack of oxygen, more closely resembles the central nervous system than it does the medullated nerve of vertebrates, seems to be reinforced by the fact that it has also a more marked retention of action current, which might serve as a basis of summation, and thus furnish a possible clue to the higher degree of cumulative effect of successive impulses in the reflex center.

SUMMARY

1. The balancing of excitatory and inhibitory central effects in the extensor center has been studied by isometric recording of contraction in the knee extensor, vasto-crureus, in the decerebrate cat. The effective strength of the inhibitory stimuli has been controlled by simultaneous recording of action currents in the ipsilateral afferent nerve.

2. Maximal stimulation of the ipsilateral (peroneal or popliteal) nerve causes complete inhibition of the strongest crossed extension reflex. Submaximal stimulation causes complete inhibition of weak crossed extension reflexes, but as the strength of the excitatory stimulation is increased, the amount of inhibition passes through a maximum and then decreases, in this respect resembling the relation found by Creed and Eccles with the crossed inhibition of flexors. Increasing the excitatory background against which the submaximal inhibitory stimulus operates, not merely adds more motor neurones to the number previously involved, but robs the inhibitory stimulus of the power to inhibit as many motor neurones as it did when acting against the weaker excitatory background.

3. Weak stimulation of the popliteal nerve, which causes inhibition against a weak excitatory background, sometimes, in the presence of a strong excitatory background, gives instead additional excitation.

4. Our observations of the rebound contraction which follows strong inhibitory stimuli, tend to support the interpretation of this phenomenon, proposed by Sherrington and Sowton, as the delayed expression of activity of excitatory fibers in the ipsilateral nerve. The threshold of these fibers is relatively high.

5. Evidence is also adduced for the presence of inhibitory fibers of high threshold in the contralateral sciatic nerve.

6. The balance between excitatory and inhibitory influences is rarely steady. One or the other usually tends to dominate.

7. The intrinsic tendency of the spinal centers to exhibit a spontaneous alternation between extension and flexion, even in the absence of proprioceptive impulses, demands some postulate other than the simple grading of opposed excitatory and inhibitory states, acting like chemical substances.

8. Evidence at present known, pointing to the view that central excitatory and inhibitory effects are qualitatively different from those associated with peripheral nerve impulses, is discussed and shown to be compatible with an interpretation based on known functions of peripheral nerve. Sherrington's theory, which assumes a "central inhibitory state" of undetermined character, requires fewer subsidiary hypotheses and is simpler in this respect, but must still be regarded as merely a working hypothesis.

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RECIPROCAL REACTION IN THE CAT AS A POSSIBLE LOCAL MECHANISM

III. The Reciprocal Reaction of the Biceps Brachii and the Triceps Brachii

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Received for publication June 5, 1930

In the previous publications on this subject (1), (2), (3), we described experiments on the reciprocal reactions of *a*, the gastrocnemius and the tibialis anterior muscles of the frog; *b*, the same in the cat, and *c*, the flexor carpi ulnaris and the extensor carpi ulnaris of the cat. Our conclusions concerning the reciprocal reaction of the biceps brachii and the triceps brachii in the cat are described in this paper. The procedure in this last group of experiments is essentially the same as that described in our previous communications. The same precautions were used here as elsewhere.

The experiments were conducted in the following order: 1. Direct stimulation of the biceps with the brachial plexus intact. 2. Direct stimulation of the triceps with the brachial plexus intact. 3. Direct stimulation of the biceps with the brachial plexus cut. 4. Direct stimulation of the triceps with the brachial plexus cut.

Experimental Procedure. In the present study cats were used and the results of our experiments were identical. The routine procedure was ether anesthesia and tracheotomy. Tracings were recorded on a rapidly revolving drum. The skin and fascia were removed thus exposing the muscles. Great care was taken not to injure too many blood vessels. Usually the hemorrhage was slight. The muscles were carefully dissected out and the tendons of insertion cut and connected by means of pieces of string leading over sensitive pulleys to two muscle levers, one for each muscle. The lever to which the tendon of the biceps brachii was attached, was after-loaded with a 20-gram weight. The lever to which the tendon of the triceps brachii was attached was not after-loaded, but loaded by the weight of the lever and 20 grams; this was necessary in order to have tension exerted continuously upon the antagonistic muscle. Fine copper wires were used as electrodes. One was tied around the tendonous insertion of the biceps and the other was inserted into the muscular part of this muscle. The two muscles were so arranged that a definite space intervened between them,

thus the contraction of one could not mechanically affect the other. The ability of the protagonistic and antagonistic muscles to contract was always determined before and several times during the experiment.

Great difficulty was experienced immobilizing the shoulder joint. After the muscles were dissected out and the tendons of insertion cut, the limb was fastened securely to the board thus avoiding mechanical factors which might influence the results.

The stimulus employed was an optimum tetanizing current. The strength of this stimulus differed with the different animals experimented upon. It was necessary to use an optimum stimulus to obtain our results, because with too weak a current the antagonist did not relax, and with too strong a current not only the protagonist and the antagonist contracted, but also other muscles of the brachium contracted. This was probably due to a spreading of the current to the other muscles.

From our results we inferred that the tension exerted upon the antagonistic muscle is as important for its lengthening (relaxation) as is the tension upon the protagonistic muscle for its shortening (contraction), therefore it was of the greatest importance to maintain continuously an equilibratory tension of the two muscles.

After obtaining tracings in which the protagonistic muscle was the biceps and the antagonistic muscle the triceps, using the technique described above, we reversed our procedure making the triceps the protagonist and the biceps the antagonist. The latter half of the experiment was accomplished in the following manner: The triceps was after-loaded with the 20-gram weight and the biceps was not after-loaded but loaded by the weight of the lever and 20 grams.

EXPERIMENTAL RESULTS. *1. Direct stimulation of the biceps with the brachial plexus intact.* Each time the biceps was stimulated directly by the electric current it contracted, and at the same time the triceps relaxed. This accords with the results obtained at the knee-joint of the frog and cat, where direct stimulation of the gastrocnemius caused its contraction and a synchronous relaxation of the tibialis anterior, and also with the results obtained at the elbow-joint of the cat where direct stimulation of the flexor carpi ulnaris caused its contraction and a synchronous relaxation of the extensor carpi ulnaris.

2. Direct stimulation of the triceps with the brachial plexus intact. Direct stimulation of the triceps caused a contraction of this muscle and a synchronous relaxation of the biceps. This agrees with the results obtained at the elbow-joint where direct stimulation of the extensor carpi ulnaris caused its contraction with a synchronous relaxation of the flexor carpi ulnaris, and does not agree with our findings at the knee-joint of the cat and the frog, where stimulation of the tibialis anterior causing contraction of this muscle did not cause a synchronous relaxation of the gastrocnemius.

We cannot explain why the reverse reaction occurs in muscles the origin of which is in or about the elbow-joint and shoulder-joint and not in muscles the origin of which is in or about the knee-joint.

3. Direct stimulation of the biceps with the brachial plexus cut. In this series of experiments the same results were obtained as when the brachial plexus was intact, indicating this phenomenon could be obtained independently of the central nervous system.

4. Direct stimulation of the triceps with the brachial plexus cut. Here also the results were the same as when the brachial plexus was intact; that is, we still obtained the reverse reciprocal reaction described under *2* above.

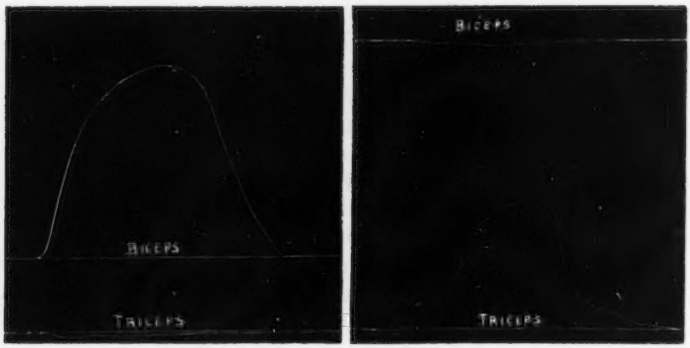

Fig. 1 Fig. 2

Fig. 1. Shows the effect of direct stimulation of the biceps with the brachial plexus cut. It will be noticed that a contraction of the biceps with a synchronous relaxation of the triceps occurs.

Fig. 2. Shows effect of direct stimulation of the triceps with the brachial plexus cut. It will be noticed here that the triceps contracts and that at the same time the biceps relaxes.

DISCUSSION. Two tracings showing our results are published. Figure 1 shows the effect of direct stimulation of the biceps with the brachial plexus cut. It will be noticed that a contraction of the biceps with a synchronous relaxation of the triceps occurs. Since the plexus is cut this result is not due to the central nervous system, but probably to some local nervous mechanism in or about the joint of the animal. Figure 2 shows the effect of direct stimulation of the triceps with the brachial plexus cut. It will be noticed here that the triceps contracts and that at the same time the biceps relaxes. This illustrates the reverse reciprocal reaction which occurs with this group of muscles as well as with the flexor carpi ulnaris and the extensor carpi ulnaris and its independence of the central nervous system.

CONCLUSIONS

1. At the shoulder-joint of the cat there appears to be a local nervous mechanism for the reciprocal reaction of the biceps and the triceps.

2. This mechanism is local in character because cutting the brachial plexus does not abolish the reciprocal reactions of the two muscles.

3. The reciprocal reactions of the two muscles in this case could be reversed, i.e., no matter which of the two muscles in question was made the protagonist, its antagonist relaxed.

Is this local mechanism a mediate agency in correlating the chronaxies of the protagonistic and antagonistic muscles?

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MATERNAL AND FETAL BLOOD SUGAR CHANGES UNDER VARIOUS EXPERIMENTAL CONDITIONS

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Received for publication May 31, 1930

The extent to which varying conditions in the maternal organism may affect the fetus has been a matter of considerable and fanciful conjecture for a number of years. Nutritional factors have been widely although inadequately considered; the effects of certain important salts have also been recognized but not thoroughly investigated. With the rapid rise in recent years in the art of the practitioner skilled in the care of pregnancy well-controlled experimentation on maternal and fetal relationships has not at all kept pace.

During a series of experiments on responses to insulin under various conditions a case arose which appeared significant and prompted further inquiry. From the protocol the following pertinent data may be cited.

Experiment VII. Cat 41; weight, 3.2 kilos. March 3, 9:07 a.m., fasting blood sugar 97 mgm. per cent. At 9:08, insulin 2 units per kilo was injected subcutaneously. The animal was observed to be in labor at 10:15. Blood samples were taken from the mother (ear vein) and from the kittens (umbilical or caudal vessels) as they were born and at intervals thereafter. Determinations were made for blood sugar, with results as follows:

Kitten number and time born	*Maternal blood sugar and time*	*Kitten blood sugar and time*
No. 1 at 10:35.....	43 mgm. at 10:54	134 mgm. at 10:50
		168 mgm. at 11:36
		163 mgm. at 1:29
No. 2 at 11:20.....	45 mgm. at 11:28	103 mgm. at 11:26
		330 mgm. at 12:31
		57 mgm. next day
No. 3 at 12:27.....	43 mgm. at 12:37	92 mgm. at 12:35
		57 mgm. next day
No. 4 at 1:25.....	67 mgm. at 1:34	90 mgm. at 1:32
	68 mgm. at 2:25	156 mgm. at 2:34

No hypoglycemic symptoms observed; all kittens were active and appeared normal.

The striking observation was made that while the blood sugar of the parturient mother may be extremely reduced following insulin administration, that of the offspring (taken just after birth) may be quite well maintained within the normal limits. There was a diminution however in the (initial) sugar levels, from kitten 1 to kitten 4, as insulin action was more prolonged. An hour or so after the kittens were born, their blood sugars were found to have risen to abnormally high levels. These points are illustrated in figure 1. Two kittens were sacrificed in drawing the blood samples and two survived.

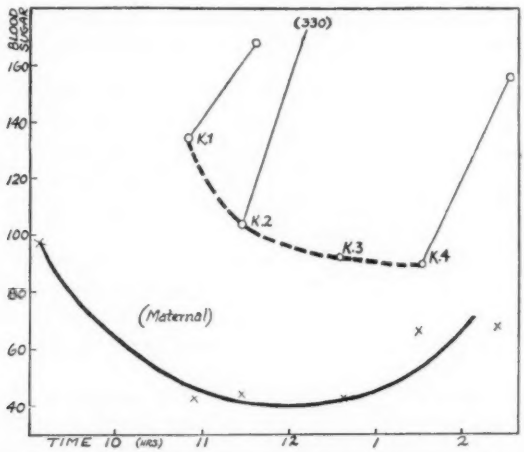

Fig. 1. Graph illustrating blood glucose levels and subsequent changes in kittens (K1–K4) born during severe but symptomless insulin hypoglycemia in the mother. Dash lines between initial values derived from the kittens indicate an interesting reactional relationship between mother and offspring. Significant increases also were noted in three kittens examined an hour after birth.

Significantly, the mother showed none of the usual symptoms of severe insulin hypoglycemia, although the blood glucose remained low throughout the period of parturition and rose only slowly thereafter. A few weeks later the same animal suffered severe insulin convulsions following the administration of the same dosage of insulin (i.e., only 2 units per kilo weight), and the blood sugar at this time fell to the very low level of 29 mgm. per 100 cc.

Careful watching of the pregnant stock animals gave opportunity for control observations to be made some time later on a cat and its litter born under normal conditions. The results in brief were as follows:

Control experiment A. April 25, 1930. Cat C1, pregnant; weight 2.8 kilos. Observed in labor at 9:15 a.m. Blood samples were drawn from mother and kittens as in experiment VII, and determinations made for blood sugar.

Kitten number and time born	*Maternal blood sugar and time*	*Kitten blood sugar and time*
No. 1 at 9:50.....	111 mgm. at 9:55	112 mgm. at 9:57
		111 mgm. at 10:58
No. 2 at 11:04.....	91 mgm. at 11:10	106 mgm. at 11:12
		133 mgm. at 12:12
No. 3 at 11:44.....	90 mgm. at 11:46	101 mgm. at 11:53
	87 mgm. at 12:55	122 mgm. at 12:49

No. 1 kitten showed only moderate activity when born; the other two were vigorously active; all appeared normal. The mother showed fatigue at end of parturition.

In the kittens at birth it will be noted that negligibly higher blood sugar values than in the mother were found. It is to be observed also that there were only relatively slight changes one hour after birth; i.e., −1, +27 and +21 mgm., in comparison with +34, +228 and +66 mgm. in experiment VII. The maternal blood sugar was lower at the end of parturition than when labor began.

When the fetuses were delivered experimentally about one week before term in another animal (C 2), there was again found a disparity between the maternal and fetal blood sugar content. This time, however, it was slightly in favor of the mother. An abstract from the protocol follows.

Control experiment B. March 31, 1930. Cat C2, pregnant; weight 2.3 kilos. 11:55 a.m., fasting blood sugar (ear vein), 81 mgm. per cent. Animal stunned at 11:57; abdomen opened, fetuses delivered. Blood sugar of fetuses, 12:000, no. 1, 72 mgm.; 12:01, no. 2, 74 mgm.; 12:02, no. 3, 72 mgm. At 12:03, blood sample from (beating) maternal heart: glucose content, 85 mgm. per cent. Fetuses were apparently within a week of time of normal delivery.

In this experiment the blood sugar of the fetus near term was observed to be slightly lower than that of the mother.

Further investigation of maternal and fetal blood sugar under various experimental conditions seemed desirable. Immediately after taking the maternal sample (ear vein) the animal in the foregoing experiment and in subsequent cases was stunned sufficiently to produce unconsciousness, and the fetuses delivered one by one (as used) through an abdominal incision. This was practised with one exception, in which amytal was used instead of stunning, under all the experimental procedures employed. Blood was taken from the jugular veins of the fetuses, and the final sample from the mother was taken from the heart. Usually the whole operation from the time of stunning to the drawing of the last blood sample occupied 4 to 6 minutes. The Folin (1928) method was used for making the analyses.

The significant data are given in table 1. Besides the series of pregnant insulinized animals which was studied (A), reactions in the mother and fetuses were followed after adrenalin injection (B), after the administration of glucose (C), and after emotional excitement (D). In all cases except one (cat 1) in group (A), insulin was given in dosage sufficient to produce severe convulsions—4 units per kilo. The animals were sacrificed after convulsions had ensued for 30 minutes to an hour or more, and usually between 4 and 6 hours after insulin injection. It was thereby hoped to develop a severe emergency situation such as would tend to affect profoundly the fetal blood sugar level.

Adrenalin and glucose were given intraperitoneally in the doses mentioned in experimental groups (B) and (C). From one to two hours afterwards in different experiments, when hyperglycemia had been well established in the mother, the fetuses were examined. In series (D), vigorous excitation of the (caged) cats by a barking dog was carried out for four minutes as indicated elsewhere (Britton, 1928).

Because of the scarcity of pregnant animals, one dog and two rats were also used in the experiments; in the case of the latter, two fetuses were necessary for each blood sample. It should be emphasized that in all cases the animals were used when they appeared to be in the later stages of pregnancy. In one instance, cat 5, prediction proved incorrect, and the embryos were found to be less than half-grown and hairless.

It will be observed from table 1 that the results fall definitely into two groupings: 1, those in which the fetal blood sugar is generally maintained at a level *higher* than the maternal, although the latter is severely reduced by insulin (A); and 2, those in which it is *lower* than the maternal when the latter is greatly increased by adrenalin or glucose administration, or by emotional excitement, in (B), (C) and (D). In two instances in the first group the results do not all appear to be in agreement, however. In one, cat 3, the fetuses were delivered after the mother had been put under amytal, and they were inactive and apparently narcotized when used; in the other case, cat 5, the fetuses were quite immature. Animal 6 was kept under insulin for a very long period—almost 6 hours—and was in convulsions for two hours before it was sacrificed, which possibly explains in part the somewhat doubtful result in this case.

It should be noted that the blood sugar of the embryo is normally lower than that of the mother at this period of pregnancy. It is the more significant therefore that under the severe experimental conditions instituted in (A), a tendency in the reverse direction becomes definitely apparent.

The results in the second group are all accordant: in several cases the fetal sugar levels are lower by about 50 per cent than the maternal values after emotional excitement or after adrenalin or glucose injection, and before stunning. This difference appears in spite of the fact, too, that

TABLE 1

ANIMAL AND NUMBER	DATE	(I) FASTING MATERNAL BLOOD SUGAR	EXPERIMENTAL CONDITIONS	(II) MATERNAL BLOOD SUGAR AT TIME OF STUNNING	BLOOD SUGAR OF FETUSES				DIFFERENCE BETWEEN MATERNAL (II) AND FETAL (AVERAGE) BLOOD SUGARS	(III) MATERNAL BLOOD SUGAR AFTER REMOVING FETUSES
					No. 1	No. 2	No. 3	No. 4		
		mgm. per cent		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
(A)										
Cat 1	5/ 9/29	92	Insulin 2 u. p. k.	67	78	74	88	97	+17	82
Cat 2	5/ 9/29	105	Insulin 4 u. p. k.	47	71	86	97		+38	90
Cat 3	5/13/29	85	Insulin 4 u. p. k.	30†	26	27	26	31	−2	44
Cat 4	6/ 3/29	130*	Insulin 4 u. p. k.	29	43	41			+13	48
Cat 5	6/13/29	74	Insulin 4 u. p. k.	28	27	28	33		+1	41
Cat 6	6/13/29	95	Insulin 4 u. p. k.	43	46	53	57	48	+8	48
(B)										
Cat 7	7/12/29	90	Adrenalin 0.04 mgm. p. k.	217	160	167	168		−72	230
Cat 8	9/25/29	98	Adrenalin 0.02 mgm. p. k.	122	79	89	104	111	−26	240
Rat A	6/21/29	108	Adrenalin 0.02 mgm. p. k.	182	80	76			−104	
Rat B	7/ 6/29	76	Adrenalin 0.02 mgm. p. k.	105	69	61			−40	103
(C)										
Cat 9	3/ 5/30	75	Glucose 0.75 gm. p. k.	121	80	72			−45	123
Cat 10	4/ 8/30	67	Glucose 0.75 gm. p. k.	121	83	79	76	77	−42	196
Dog X	3/24/30	87	Glucose 0.75 gm. p. k.	92	60	61	64	65	−30	86
(D)										
Cat 11	3/17/30	127*	Excited by dog 4 min.	184	78	88	89		−99	227
Cat 12	4/ 2/30	85	Excited by dog 4 min.	118	88	95	95	94	−25	111

* Cat excited when blood drawn.

† Blood sugar after animal had been amytalized.

the final readings of the mother's blood sugar, after stunning and delivery, usually showed noteworthy increments over the previous determinations, undoubtedly due to pre-mortal discharge from the liver. Thus, the last blood samples from the mother showed in many cases about twice the glucose values of those taken from the fetus within one minute previously.

Discussion. It was the conception of Harvey (1651) that the placenta elaborated from the maternal blood the aliment necessary for growth and development of the fetus. While it appears undoubted that under normal conditions the nutrition of the fetal tissues is maintained as if they were an integral part of the maternal organism, it also appears from the foregoing experiments that a remarkably selective or controlling ability may be exercised by either the placenta or the intra-fetal mechanisms under certain eventualities.

That the fetal blood sugar is normally much lower than that of the mother until near term, when the amounts are practically the same, has been demonstrated by Snyder and Hoskins in the rabbit (1928) and by Corey in the rat (1930). Results of the present experiments are in agreement. The former investigators also noted that the fetal liver and body glycogen values were remarkably augmented during the latter third of pregnancy. The amino-acid nitrogen and total non-protein nitrogen were shown to be present in greater concentration in fetal than in maternal blood, although no differences were detected in urea nitrogen (Snyder and Hoskins, 1929).

In the newborn (man) the glucose in the blood, and also the amino-acid nitrogen, have been found by Slemons (1919) to be higher in a large number of cases than in the mother. Further observations on the conditions found in man have been recently reported by Dahl (1928) and by Naeslund (1928).

Whereas no diabetes followed complete pancreatectomy in pregnant bitches if operated on when the fetuses were at full term, Carlson and Drennan (1911) found that in early pregnancy the usual diabetic symptoms did appear after the operation. The likelihood that the internal secretion of the fetal pancreas might explain the results has been expressed by Carlson. Evidence which has been furnished by other workers (Forschbach, 1908; Pack and Barber, 1928) corroborates this possibility. Opposition to the view that insulin might pass from the fetus to the mother has nevertheless been brought forward by Allen (1921). Furthermore, the experiments of Hoskins and Snyder (1928) give no support to the belief that endocrine substances—insulin, adrenalin, pituitrin, parathyroid extract—pass from the fetus to the mother across the placental boundary.

From the recent results of Allumbaugh (1929) it has been suggested that the placental barrier may operate to conserve important elements in the blood—hemoglobin, red and white corpuscles— for either the mother or the fetus. It is well to observe, however, that very significant variations in the concentration of hemoglobin and in the number of cellular elements

occur in the fetus at different age-periods. The more recent observations of Zeidburg (1929) and of Kindred and Corey (1930) particularly emphasize these changes and their relationships. In the evaluation of the investigative work on fetal and maternal conditions, the importance of recognizing these variations and also the normal changes in glucose and glycogen in the organism as mentioned above—all fundamental phenomena which are probably referable to enhanced metabolic activities and to the growth of the fetus—must readily become evident.

In early pregnancy, when the placenta forms a relatively thick barrier, blood sugar changes appear to be readily effected in the fetus. In the later stages of gestation, however, fetal glucose variations from the normal are brought about (and then slightly) only after severe maternal disturbance, although at this time the placenta is relatively thinner and indeed somewhat atrophic. There is indication therefore that certain intra-fetal adjusting or equilibrating mechanisms are operative at this time. Whether the maternal blood sugar content be diverted upward or downward, the fetal value consistently tends to remain within normal limits.

The release of the fetus by birth from a maternal hypoglycemic influence resulted very interestingly in rapid and marked elevation of the glucose percentage in the offspring within an hour after delivery—representing perhaps a type of rebound response. Opportunity has not yet presented itself to ascertain whether a commensurate blood sugar decrease would occur in the new-born following the production of hyperglycemia in the parturient mother. It at least seems altogether likely that very specialized functions, tending to preserve the organism, are performed by the embryonic tissues during the later stages of gestation.

SUMMARY

Comparison has been made in different animals (chiefly cats) of maternal and fetal blood sugar changes under various conditions. Under insulin the blood sugar in the mother falls readily to the convulsive level; concurrently in the fetus the glucose percentage is notably higher, however, and it may be within normal limits. Even when convulsions are allowed to extend for two hours, the sugar values are slightly in favor of the fetus. The conditions do not hold in very young fetuses, or when the mother is anesthetized before making the (Caesarean) delivery.

Normally in the later stage of pregnancy, which was the period particularly studied, the fetal blood sugar is lower than that of the mother. At birth the values in the kitten were somewhat higher than those of the mother.

In one case in which an animal gave birth to four kittens during severe insulin hypoglycemia, the blood sugars in the latter were within the normal limits, but considerable increments were observed an hour after birth. The

mother showed none of the usual hypoglycemic symptoms during parturition, although convulsions followed the same insulin dosage a few weeks later.

Marked hyperglycemic reactions which were produced in the mother by emotional excitement, or by adrenalin or glucose administration, did not affect notably the fetal glycemia.

It is indicated that during the later stages of gestation, important adjustments are able to be carried out by the fetus in response to various emergency conditions that may be imposed on the mother.

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A STUDY OF THE ISLANDS OF LANGERHANS IN VIVO WITH OBSERVATIONS ON THE CIRCULATION

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Received for publication June 18, 1930

The earliest recorded observations concerning the appearance of the islands of Langerhans in a living animal were made by Kühne and Lea in 1882 (1). These investigators described the islands in the pancreas of the living rabbit as small opaque spots resembling tapioca grains and having the microscopic appearance of glomeruli containing numerous capillaries. Their observations were fragmentary and incomplete due to the technical difficulties they encountered. In 1899 (2) Mathews also attempted to study the pancreas in the living animal but made no new observations. Covell (3) in 1928 used the living white mouse to study the changes in the acinar cells of the pancreas during secretory activity. In 1930 while this work was in progress there appeared a paper by O'Leary (4) who adopted Covell's method to study the islet cells in vivo.

The following report deals with observations concerning the physiology of the islands of Langerhans in the pancreas of a living mammal, and includes a description of the circulation with its reactions to different drugs. After an examination of the pancreas in various species of commonly used laboratory animals, it was found that the pancreas of the white mouse was the most satisfactory for this purpose. The methods that were employed in this investigation were similar to those used by Richards (5) in his observations on the glomerular circulation in the frog's kidney, and were based upon Krogh's (6) studies of the capillaries.

TECHNIQUE. White male mice weighing from 10 to 20 grams were used. Anesthesia was induced by the subcutaneous injection of 1 mgm. of sodium amytal (Eli Lilly & Co.) per 5 grams of body weight. After 10 to 20 minutes narcosis was complete. The animal was placed upon its right side in a petri dish that rested upon a warm stage. An incision measuring 1 cm. was made in the left flank, and the anterior pole of the spleen was grasped and drawn through the opening in the abdominal wall. By gentle traction and rotation, the tail of the pancreas was brought into view, attached to the hilum of the spleen. After teasing the lobules apart to expose the main

central vessels, the pancreas was bathed with physiological saline at 37°C. and was covered with a small cover slip. Observations for periods extending from one to two hours were made with a Leitz binocular UBM type of microscope at a magnification of 75 and 96 times using filtered direct illumination.

OBSERVATIONS. In suitable preparations, the islands could be identified by the naked eye as minute white dots on a yellow background. They were distributed usually along the course of the main central vessels or branches of the latter. Microscopically, they appeared as brilliant yellowish white bodies on the surface of the less refractile yellow pancreas or on a vein. They were round, oval or kidney-shaped, had well defined margins and varied from approximately 0.6 mm. to 0.07 mm. in diameter. No spontaneous changes in the number, size or shape of the islands were noted. Islands that were studied in vivo were identified by serial sections.

Accurate observations were possible only when the islands were situated at the surface of the pancreas, although when they were imbedded deeper in the gland, they could be distinguished by the fact that they reflected the light more brilliantly than did the surrounding tissue. Great anatomical variations existed in different animals with respect to the number of islands that were superficial. In the glands of some mice, many islands were found at the surface. In others, only one or two were available for study. In some, no islands were found at the surface. The so-called "interstitial islets" of Bensley (7) were found to be most suitable for observation.

The circulation in the islands was distinguished clearly. The capillaries appeared as thin, red thread-like loops that had U, S, V, or spiral shapes and dipped into the substance of the islands. The capillaries were situated between interlacing double columns of highly refractile islet cells. After applying a few drops of warm saline the capillaries became more clearly visible; this effect lasted for thirty to sixty seconds. The number of visible capillaries varied in different glands. Sometimes, no vessels were seen. When the capillaries did not contain a sufficient number of erythrocytes, they appeared as grey translucent channels between double rows of secretory islet cells. As a rule, the main capillary pattern remained unaltered, although lightning-like changes were observed frequently, now one, now another capillary becoming visible and then disappearing from view. The circulation was extremely rapid and the red blood cells were not distinguished. The direction of the flow of blood was often noted in the efferent venules at the periphery of the islands. In this region, changes in the course of the flow of blood through different capillaries occurred at irregular intervals. Anastomoses between the capillaries of the islands and the interacinar capillaries were seen. In a few instances, the afferent arterioles were distinguished, but as a rule they were not visible. Rhythmic spon-

taneous contractions were observed on a few occasions in the small arteries. In studying the circulation, it was important to determine whether any pancreatic tissue was interposed between the island and the surface because the interacinar capillaries were easily confused with those in the underlying islands. Due to the opacity of the islands, observations concerning the circulation were restricted to capillaries close to the surface. Confirmatory anatomical studies concerning the arrangement of the circulatory system

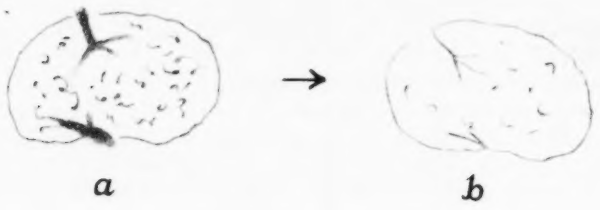

Fig. 1. Blanching of island of Langerhans 30 seconds after the intravenous injection of 0.03 cc. of pituitrin in 1:10,000 dilution. Magnification 100×.

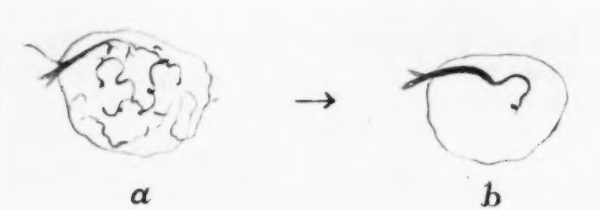

Fig. 2. Blanching of island of Langerhans and stasis in efferent venule 30 seconds after the intravenous injection of 0.01 cc. of epinephrin in 1:100,000 dilution. Magnification 100×.

in the islands of Langerhans were made and will be reported in another paper (8). It was found that one or more afferent arterioles and one or more efferent venules were connected with each island; in addition, anastomoses existed between the capillaries in the islands and those in the adjacent acinar tissue. These findings corresponded with the observations made in vivo.

The variations in the capillary pathways which were observed in some

of the islands were due probably to changes in the proportion of red cells to plasma in the circulating blood, resulting from alterations in the afferent arterioles. The anatomical arrangement of the capillaries did not favor independent contractility. Although intermittence of the circulation in the islands was not observed, the possibility of its existence could not be excluded because the number of islands that were available for study in any one preparation was limited.

Effects of vasoconstrictor drugs. Observations were made concerning the action of epinephrin and pituitrin on the circulation in the islands of Langerhans. In addition, the reactions of small arteries (approximately 0.25 mm. in diameter) and arterioles (approximately 0.10 mm. to 0.25 mm. in diameter) were also studied. The drugs were injected slowly into the left lateral tail vein by means of a 27 gauge needle attached to a tuberculin syringe. The dosage varied from 0.01 cc. to 0.1 cc. according to the concentrations employed. Both epinephrin and pituitrin caused blanching of the islands in dilutions of 1:100,000. Within 15 seconds to 30 seconds after the injections, marked pallor of the islands developed. The capillaries disappeared from view or were outlined as narrow empty channels lying between double columns of secretory cells. Sometimes a few capillaries and efferent venules remained visible due to venous stasis. The small arteries and arterioles responded to epinephrin in dilutions as high as 1:10,000,000, and to pituitrin in dilutions of 1:1,000,000. The duration and intensity of the vasoconstrictor effects varied inversely with the order of dilution.

SUMMARY

The capillary circulation in the islands of Langerhans can be observed in vivo in the pancreas of the white mouse. Variations in the circulation are due to changes in the afferent arterioles. Intravenous injections of epinephrin and pituitrin cause blanching of the islands by vasoconstrictor action on the arterioles.

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AN EXPERIMENTALLY PRODUCED PREMATURE SYSTOLIC ARHYTHMIA (PULSUS BIGEMINUS) IN RABBITS

II. Secondary Factors Which do not Produce It

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Received for publication July 15, 1930

It has been shown by Kisch, Hering, C. Heymans (4), (5) (6) and Régniers that ectopic beats may sometimes be caused from occlusion of the carotid artery and from hypertension in the cephalic end of this vessel. Kisch attributes this change to the rise in blood pressure and to anemia in the medulla. Hering holds the arhythmia to be due to a carotid sinus reflex. Régniers demonstrated by an electrocardiogram that ectopic beats can be obtained in rabbits from occlusion of the carotid artery after denervating the carotid sinus and sectioning the depressor nerve to the aorta. According to this author the production of ectopic beats by occlusion of the carotid artery is favored by sectioning the depressor nerves, not inhibited by sectioning the vagi and notably diminished through injection of ergotamine. By use of the isolated head technique in dogs, Heymans and Heymans (3) have shown that cephalic and peripheral hypertension and central and peripheral anemia are factors which induce or reënforce the so-called respiratory arhythmia. There is no end of literature relating to pulse alteration caused from physical and chemical vascular changes.

It is important therefore at this stage of the problem to determine if the bigeminal pulse described in the first paper of this series as being elicited from certain sensory stimulations, is not actually due to some secondary agency resulting from these stimulations, the most suggestive of which are: 1, asphyxia; 2, mechanical stimulation or lack of stimulation from the arrest of respiration; 3, sudden changes in blood pressure and pulse; 4, anemia in the brain stem; 5, certain humoral changes such as increased secretions of the adrenals, thyroid, liver or the formation of a hormone. A study of these agencies will form the basis of this paper. The possibility of the carotid sinus being an indirect afferent center for producing this arhythmia will receive special attention.

It was shown in the previous paper that a bigeminal pulse evoked from insufflation of benzol or from other means was usually preceded by an

arrest of respiration, a rise in blood pressure and a slowing and strengthening of the pulse. It was also noted that these changes were almost nil in a few records.

Asphyxia. Direct asphyxia and toxemia from the insufflations are sufficiently excluded as agencies for eliciting the bigeminal pulse because respiration always took place through a tracheal cannula. It was shown in the previous paper that inhaling (not insufflating) strong concentrations of ammonia and bezol vapor through a tracheal cannula or a slit in the trachea did not produce an arhythmia in several rabbits which readily gave this arhythmia from insufflation or inhalation through the nostrils. Attention is here directed to the fact that the same bigeminal pulse was also easily obtained in many rabbits in the previous study from electrical and mechanical stimulation of the nasal septum and from faradic stimulation of the central end of the vagus nerve.

A considerable number of asphyxia tests were carried out in six rabbits which readily yielded a bigeminal arhythmia from benzol insufflation before and after the tests. Anoxemia was produced by occlusion of the trachea or tracheal cannula for intervals considerably longer than the time of arrested respiration during an insufflation test. Apical beat and carotid pressure tracings were recorded after the manner described in the previous paper of this series.

One of these records (fig. 1 B of the previous paper) exhibits only the rise in blood pressure and the gradual slowing and strengthening of the pulse that is characteristic of early anoxemia. In another record where the trachea was closed for 2 minutes and 24 seconds, the crest of the blood pressure rise occurs in 1.5 minutes. At this stage the pulse rate is reduced one-half and the height of the pulse waves has increased three times. None of these records show a bigeminal pulse during or after the period of suppressed ventilation.

Two rabbits in which a bigeminal pulse was easily produced from mechanical stimulation of the nasal septum or benzol insufflation were required to breathe carbon dioxide or air highly concentrated with it for intervals varying from 20 seconds to 2 minutes. Numerous carotid tracings taken at the time and following these inhalations reveal the total absence of a bigeminal pulse. Typical apex beat and carotid tracings from a rabbit which inhaled air highly concentrated with carbon dioxide from a tank for 22 seconds show a greatly deepened respiration, a gradual drop in blood pressure of 19 mm., a reduction in the height of the pulse waves by one half but no change in their rate. Longer inhalations of carbon dioxide caused the gradual dropping out of every second beat without any suggestion of a bigeminal pulse.

Discontinuance of respiration. The following experiment was devised to determine whether this arhythmia could be elicited with respiration

taking place regularly without intrathoracic changes. Three rabbits were given artificial respiration at a rate slightly below their normal rate of breathing to which they readily adjusted their own respiratory movements. This was shown by the synchronous movements of the nostrils and the pulmotor. The thoraces of these animals were opened for their entire width on both sides between the third and fourth ribs. In one of these animals a bigeminal pulse followed the second mechanical stimulation of the nasal septum and with the other two it came on with the first and third insufflation of benzol.

Figure 1 A is a bigeminal pulse record from one of the double pneumothorax rabbits following benzol insufflation. As is to be expected, the upper respiratory tracing exhibits no change during the insufflation period. It was observed that the nostrils were also not perceptibly inhibited during the insufflation period and continued to follow the movements of the pulmotor. The carotid tracing below reveals a bigeminal pulse beginning at the peak of the blood pressure rise. The omitted portion of this record (a 20 second interval) shows a bigeminal pulse throughout, and the return to normal appears at the extreme right.

In several rabbits where the blood was well oxygenated by artificial respiration and the pulmotor stopped, there was an apnea period of considerable duration and no bigeminal pulse.

Sudden changes in blood pressure and pulse. Since a number of investigators have obtained extrasystoles in dogs and rabbits from increased blood pressure, especially in the carotid sinus, it is obligatory in this problem to determine if the sudden and pronounced rise in blood pressure which usually precedes a bigeminal pulse induced from insufflation, mechanical stimulation of the nasal septum or faradic stimulation of the central end of the vagus, is not in some way the actual cause of the arhythmia. With this in mind, a number of sudden rises in blood pressure were brought about through clamping off certain large arterial trunks and from adrenalin injected intravenously. In these tests the rise in blood pressure ranged from the same height to considerably higher and lasted for the same time or longer than was the case in the insufflation tests.

The abdominal aorta was pressed against the vertebral column 8 to 15 times for 15 seconds to 2 minutes in six rabbits which had yielded a bigeminal pulse readily from benzol insufflation. Carotid tracings taken during and following these tests show no arhythmia. The main or upper carotid tracing of figure 1 B is representative of a rise in blood pressure from occlusion of the aorta, in which the curve, though considerably higher, closely resembles the rise in blood pressure that usually precedes a bigeminal pulse in an insufflation tracing.

The lower insert tracing of figure 1 B is the first part of a carotid tracing taken during the second compression of the abdominal aorta in rabbit 748.

It illustrates the slowing and strengthening of the pulse which frequently occurs in a rise in blood pressure from occlusion of the aorta. The aorta was compressed fifteen times in this animal for 15 seconds to 25 seconds

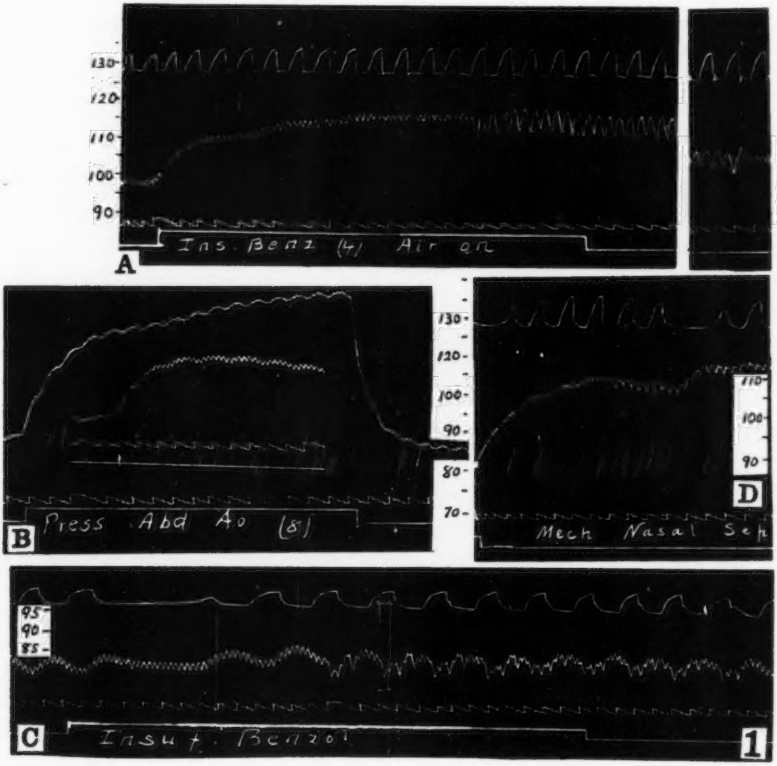

Fig. 1 A. Thoracic respiratory tracing above, carotid, below, during benzol insufflation from a rabbit breathing by artificial respiration and thorax opened on both sides, time in seconds.

B. Carotid tracings from two different rabbits at the time abdominal aorta was compressed. Lower tracing includes only the first part of the record to show pulse changes.

C. Thoracic respiratory and carotid tracings at the time of benzol insufflation from a tracheotomized rabbit, the spinal cord of which was severed at the level of the seventh thoracic vertebra.

D. Thoracic respiratory and carotid blood pressure tracings at the time of mechanical stimulation of the nasal septum of a rabbit which had both carotid sinuses extirpated.

and once for 2 minutes. Many of the resulting blood pressure rises (see fig. 1 B) are indentical in every way to the rises evoked from insufflation. Each rise in blood pressure was followed by a compensatory rise which was fully as high as the original rise, but none of these thirty rises produced a bigeminal pulse.

Three occlusions of the abdominal aorta in a dog failed to produce an arhythmia.

Fifteen and twenty femoral blood pressure tracings taken from two rabbits at the time the right carotid artery was compressed from 15 seconds to 3 minutes show no sign of a bigeminal pulse. In general these tracings portray a slow rise in blood pressure amounting to 10 or 12 mm. and no perceptible pulse changes.

Six rabbits which had given a bigeminal pulse following insufflation of benzol and were in condition to produce one, were given two or three injections of 0.2 to 0.25 cc. of 1:1000 adrenalin into the jugular or ear veins at intervals of about 15 minutes. Carotid tracings following these injections demonstrate only the pronounced rise in blood pressure and pulse changes that are characteristic of this drug. The second injection of adrenalin produced a rise in blood pressure of 70 mm. in rabbit 701, with no suggestion of an arhythmia.

Several injections of adrenalin in two dogs gave the typical rise in blood pressure and no arhythmia.

It is obvious that all of the many asphyxia tracings produced from occlusion of the trachea bear evidence against changes in pulse and blood pressure being the direct cause of the bigeminal pulse following insufflations of benzol. In these records you have most pronounced slowing and strengthening of the pulse, some rise in blood pressure, but no arhythmia.

Experimental conditions in which a bigeminal pulse follows benzol insufflation without blood pressure changes. In the previous paper of this series a few carotid tracings were described in which a bigeminal pulse followed insufflations of benzol or mechanical stimulation of the nasal septum with very little preceding changes in the blood pressure.

Figure 1 C is a carotid tracing of the first bigeminal pulse obtained by benzol insufflation from a rabbit in which the spinal cord was sectioned at the level of the seventh thoracic vertebra one week before these tests were made. This was confirmed at autopsy. Previous to making these tests the animal was given a three-fourth anesthesia dose of veronal sodium and under light ether the inferior cervical ganglia were removed and the vagus and depressor nerves were cut on both sides at the level of the larynx. As a result of one of the benzol insufflations the carotid tracing (fig. 1 C) shows a bigeminal pulse (type 2 variety), which is preceded by almost no rise in blood pressure. The pulse is retarded and strengthened and respiration is arrested for a short interval prior to the appearance of the arhyth-

mia. It is obvious that the arhythmia of this tracing contains one or more normal beats intercalated between several bigeminal waves. Other rabbits in which the spinal cord had been cut at approximately the same level have likewise yielded bigeminal pulse records from benzol insufflation which were preceded by little or no rise in blood pressure.

At the suggestion of Prof. C. Heymans a number of rabbits were given ergotamine intravenously for the purpose of preventing an arhythmia from benzol insufflations by blocking the sympathetic impulse to the heart. This was not accomplished with the doses given, although it may have de-

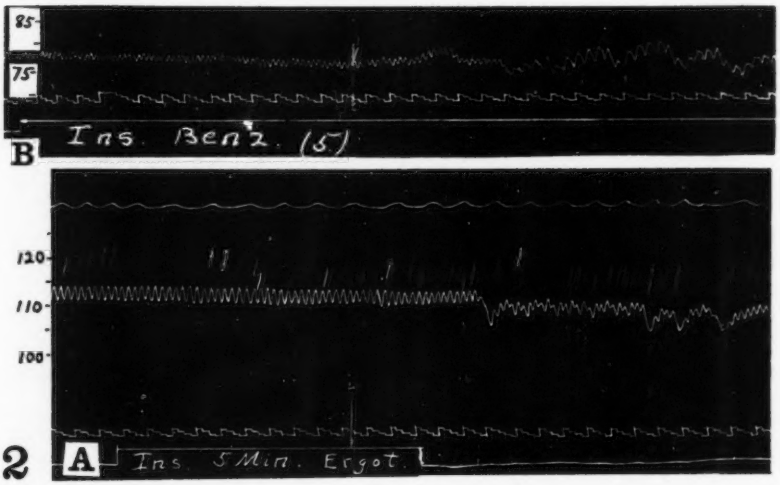

Fig. 2 A. Thoracic respiratory and carotid tracings from a tracheotomized rabbit taken during benzol insufflation 5 minutes after an intravenous injection of 0.5 mgm. of ergotamine per kilo, time in seconds.

B. Carotid tracing from a tracheotomized rabbit having abdominal aorta connected to a blood pressure equalizer at the time of benzol insufflation.

layed the coming on of the arhythmia and possibly shortened its duration in some instances. Apparently the drug acted on the peripheral nervous system to the extent of preventing a rise in blood pressure from insufflations, thus permitting an insufflation of benzol to produce a bigeminal pulse without any change in blood pressure.

The results obtained with ergotamine however were not uniform. In some rabbits a 0.25 mgm. per cent solution in water per kilo weight would prevent a rise in blood pressure and in other rabbits 0.5 mgm. per kilo weight permitted considerable rise in blood pressure.

Blood pressure and respiratory tracings were obtained after the manner

described in the previous paper. A rabbit was given 0.25 or 0.5 mgm. of ergotamine per kilo weight intravenously. Insufflations of benzol were dispensed 2, 3, 5, 8, 10, 15, 20 minutes and sometimes 1 and 2 hours after the drug.

The lower carotid tracing (fig. 2 A) is representative of several from five rabbits which demonstrate how the usual rise in blood pressure following insufflation is apparently prevented or greatly reduced by ergotamine and a bigeminal pulse follows the insufflation. This rabbit was given 0.5 mgm. of ergotamine in aqueous solution per kilo weight and the record is from the third insufflation of benzol administered five minutes after the injection. It is obvious that the bigeminal pulse (a type 2 variety) occurs without any previous rise in blood pressure. The pulse likewise shows little or no change preceding the arhythmia, but respiration is inhibited.

An attempt was made in two rabbits to regulate blood pressure by an equalizer designed by Professor Greene and the writer is under great obligations to Doctor Greene for a sketch of this apparatus. In the experiment the equalizer was connected to the abdominal aorta at its division into the iliacs and blood pressure was recorded from the right carotid. The height of the overflow spout of the equalizer was arranged so that it would be a little below the level of the average rabbit's blood pressure. It was expected that the animal's blood pressure would not fluctuate after the above connections were established, but there seemed to be a tendency to change. Frequently a drop would occur immediately before an insufflation so that the carotid manometer would record a slight rise from the insufflation stimulation.

The carotid tracing in figure 2 B however was caught at the right time. Here an insufflation of benzol has produced a bigeminal pulse (type 2 variety) without being preceded by a rise in blood pressure. A slight rise in one place during stimulation is due to the first inspiration after the arrest of respiration.

Humoral factors. It has not been demonstrated thus far that the arhythmia impulse induced from one or more stimulations of certain cranial nerves does not first produce certain internal secretions which might be directly or indirectly responsible for this arhythmia. The most suggestive of such secretions are those from the adrenal, thyroid and liver or some vascular hormone. Epinephrin can be presumptively excluded as a factor from an earlier negative experiment in which adrenalin was injected intravenously to raise blood pressure. Also it might be expected, if the arhythmia is due to secondary humoral causes, that stimulation of the lingual branch of the trigeminal nerve would as readily bring it about as stimulation of the maxillary branch. In order that humoral factors might be eliminated from this problem, cross circulations were established between two rabbits and a bigeminal pulse was produced in one animal from insufflation of benzol.

The procedure consisted of selecting two rabbits of about the same size or the "arhythmia rabbit" being slightly the larger. Generally the blood of the two was matched, for which the writer is obligated to Miss Strube of the Department of Bacteriology. Both rabbits were given a little less than an anesthesia dose of veronal sodium intravenously in divided doses. Sufficient heparin was also injected intravenously in divided doses to prevent clotting of the blood. After strapping the two animals side by side to the same animal board, the outer common carotid of each animal was connected to a mercury manometer, so placed that the blood pressure of rabbit B would be recorded directly above that of rabbit A. A cross circulation was established from A to B and B to A through connection of the juxtaposed carotids. This was accomplished in every case by connecting a short cannula to a specially made z-shaped cannula. All cannulae were filled with normal saline or Ringer's fluid from a hypodermic syringe. After the head clamps had been removed, all that remained to establish crossed circulations was to remove two carotid clamps which had been placed between the z-shaped cannulae and the hearts. Sometimes this was done before the arhythmia was elicited in rabbit A and sometimes at its appearance. A number of preliminary tests demonstrated that establishing a cross circulation, after the manner described, never of itself produced a bigeminal pulse.

Tracings A and B (fig. 3) are illustrative of three other cross circulation experiments which indicate that there are no products in the blood of an animal at the time of this arhythmia which will produce a like arhythmia in a second animal.

The carotid tracing of rabbit A (fig. 3 C, A) shows a bigeminal pulse of the type 1 variety evoked from the first mechanical stimulation of the nasal septum. It came on at the peak of a 26 mm. rise in blood pressure. As soon as possible after this arhythmia was observed in rabbit A (see arrow fig. 3 C) both clamps were released and the carotid blood gushed from A to B and from B to A. Observe that there is absolutely no sign of a bigeminal pulse in the carotid tracing of rabbit B, either at the time of the arhythmia or directly after (fig. 3 C) or 1 minute later (fig. 3 D), 2 minutes later (fig. 3 E) or 12 minutes later (fig. 3 F). The omitted portions of the carotid tracings A and B likewise exhibit no sign of a bigeminal pulse. Immediately after completing this test, rabbit B yielded a bigeminal pulse (fig. 3 G) from the first mechanical stimulation of the nasal septum, showing that this animal was undoubtedly in condition to yield an arhythmia if arhythmia producing substances were present in the blood of rabbit A.

Two of the other three cross circulation experiments were fully as decisive as the one just described, except that the interval of transfusion lasted 3 to 6 minutes instead of 12 minutes. In one of these experiments

(694) two bigeminal pulse arhythmias of type 1 variety were produced in rabbit A from mechanical stimulation of the nasal septum. The interval

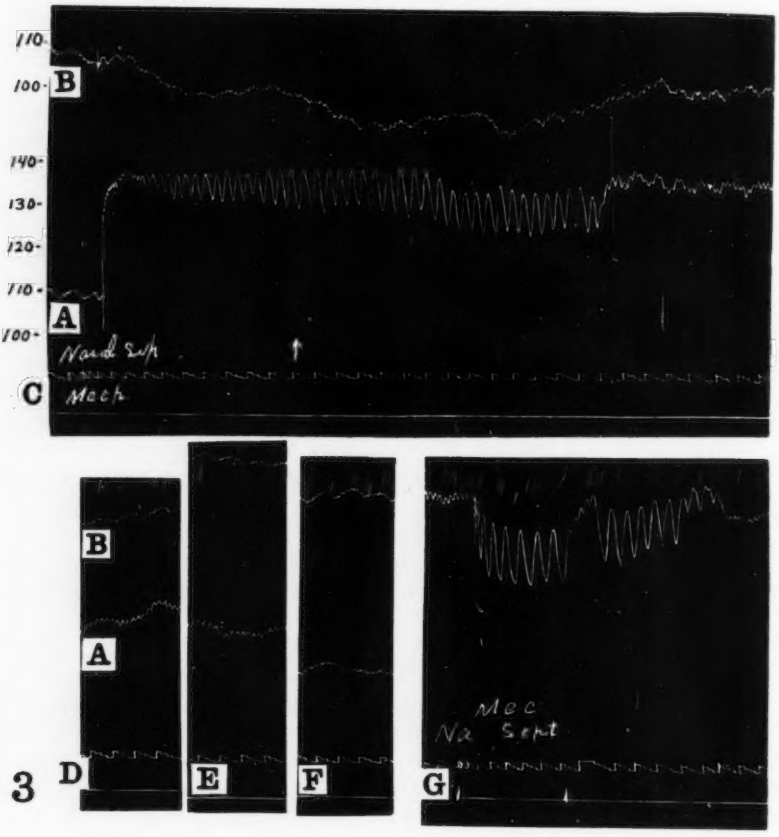

Fig. 3. Cross carotid circulation experiment—A, carotid tracing from a tracheotomized rabbit in which a bigeminal pulse was produced from mechanical stimulation of the nasal septum. B, carotid tracing from rabbit B. Arrow signifies time cross circulation was established. C, carotid tracings A and B at time of arhythmia in A; D, one minute later; E, two minutes later; F, twelve minutes later. G, a bigeminal pulse produced in rabbit B from mechanical stimulation of the nasal septum 2 minutes after F.

or double carotid transfusion lasted for 3 and 6 minutes respectively. There is no sign of an arhythmia in either of the carotid tracings of rabbit B taken during or after the arhythmia in rabbit A. In the other experi-

ment (692) the rabbits were of identical size and blood pressure. The cross circulations were established at the beginning of the experiment and carotid tracings were taken after a bigeminal pulse (type 1 variety) had been evoked first in one animal and then in the other from benzol insufflation. As before, the carotid tracings demonstrate that the arhythmias, three in number, are not carried over from one rabbit to the other through an exchange of blood.

The first crossed circulation experiment was not a complete success due to faulty carotid connections between rabbit B and rabbit A. A bigeminal pulse was readily obtained in rabbit A from benzol insufflation, during which considerable blood passed from A to B without eliciting any arhythmia in rabbit B. A second bigeminal arhythmia was produced in rabbit A from insufflation of benzol, and while the arhythmia was in progress—70 cc. of blood were withdrawn from the carotid artery of A and injected into the jugular vein of B. This was completed within a minute and a carotid tracing taken from rabbit B during and after the injection shows no arhythmia.

Central anemia. It is obvious that the change in blood pressure following benzol insufflation and mechanical stimulation of the nasal septum is not contributory to anemia of the central nervous system.

Carotid sinus. The preceding experiments, where no arhythmia was obtained from identical and considerably more pronounced rises in blood pressure produced from occlusion of the aorta and adrenalin, together with others where the arhythmia was obtained from insufflations without any preceding rise in blood pressure, all bear evidence against the carotid sinus being the source of the stimulus which produces the arhythmia following insufflations and mechanical stimulation of the nasal septum.

The following tests show that a bigeminal pulse may take place readily from benzol insufflation without any assistance from the carotid sinus.

In three rabbits the carotid sinuses were denervated, both common carotids were ligated 20 mm. below their bifurcation and both external and internal carotids were ligated 8 to 10 mm. above the division of the common trunk. Femoral blood pressure tracings demonstrate that the bigeminal arhythmia came on with the first mechanical stimulation of the nasal septum in one animal, with the first insufflation of benzol in the second animal and with the fourth insufflation of benzol in the third animal.

Upon failure to obtain a bigeminal pulse in rabbit 748 from 15 compressions of the abdominal aorta, the carotid sinuses were completely removed after severing both common carotids 20 mm. below their bifurcations and sectioning both external and internal carotids 10 mm. above their source from the common carotids. Bigeminal arhythmias were elicited from the first mechanical stimulation of the nasal septum (fig. 1, D) and the first insufflation of benzol.

Some attempts to stop the bigeminal arhythmia. Pinching one vagus and cutting both. during the arhythmia, did not produce a normal rhythm in two rabbits. Intravenous injections of atropin however met with much better success as shown by the following tests.

After having obtained a bigeminal pulse several times in two rabbits which had both vagi and depressors cut at the level of the larynx and both inferior cervical sympathetic ganglia removed, benzol insufflation finally brought on an arhythmia which continued for at least 15 or 20 minutes. The bigeminal pulse of the first animal was stopped instantly upon the receipt of 1 cc. of 1 per cent atropin intravenously and subsequent insufflations of benzol failed to evoke the arhythmia. A normal rhythm was obtained for 30 seconds in the second arhythmia animal from an intravenous injection of 0.5 cc. of 1 per cent atropin. Shortly after the bigeminal pulse returned, an additional injection of 1.5 cc. of 1 per cent atropin instantly stopped the bigeminal rhythm and several insufflations of benzol failed to elicit another or any appreciable pulse changes.

Two 2.5 kilo rabbits which readily yielded a bigeminal pulse from benzol insufflations ceased to respond after having been given atropin intravenously. The most sensitive of these two rabbits gave a bigeminal pulse from the first mechanical stimulation of the nasal septum. After injecting 1.25 cc. of 1 per cent atropin into an ear vein, insufflations of benzol administered 2, 5 and 8 minutes later showed short intervals of bigeminal pulse following the first and last insufflations. They came on slowly and apparently with difficulty. A second injection of atropin consisting of 2 cc. of 1 per cent was followed by insufflations of benzol 2, 4, 6, 10, 10.5, 20 and 47 minutes later without evoking an arhythmia. Carotid and respiratory tracings during these insufflations show considerable rise in blood pressure, some slowing and strengthening of the pulse (reduced considerably by the atropin) and respiration is always arrested.

A 3 kilo rabbit which had the vagus and depressor nerves cut on both sides and both inferior cervical ganglia extirpated, yielded a bigeminal pulse with the first insufflation of benzol, but after injecting 1.5 cc. of 1 per cent atropin into the jugular vein, insufflations of benzol 2, 8, 10 and 12 minutes later failed to produce the arhythmia.

SUMMARY AND CONCLUSIONS

Closure of the trachea and inhalation of air highly concentrated with carbon dioxide for the same and much longer periods than are represented by the arrest of respiration in the insufflation tests have not produced a bigeminal pulse.

Apnea following forced artificial respiration has not evoked a bigeminal arhythmia.

A bigeminal pulse is readily obtained from benzol insufflation in rabbits having a double pneumothorax and artificial respiration.

A bombardment of sudden rises in blood pressure from compression of the aorta or adrenalin has not effected a bigeminal pulse. These rises in pressure were identical or more pronounced and of longer duration than the rise following benzol insufflation.

A bigeminal pulse is elicited from benzol insufflation in rabbits in which blood pressure was prevented from rising—*a*, by use of an equalizer; *b*, by ergotamine; *c*, by sectioning the spinal cord in the middle thoracic region.

Asphyxia from occlusion of the trachea causes a more pronounced pulse than insufflation of benzol and there is no arhythmia.

Many bigeminal pulse arhythmias have been obtained from benzol insufflation without any preceding alteration of the pulse.

The normal reaction of blood pressure to benzol insufflation is not one generally associated with the production of anemia in the brain.

Crossed circulation experiments show that there are no substances in the blood of a rabbit having an experimentally produced bigeminal pulse which will call forth a similar arhythmia in a second rabbit.

Stimulation of the carotid sinus by the above listed mechanically produced rises in blood pressure has not caused a bigeminal arhythmia.

A bigeminal arhythmia is easily obtained from benzol insufflation or mechanical stimulation of the nasal septum in rabbits having their carotid sinuses extirpated or the sinuses denervated and ligated above and below.

It would appear then that asphyxia, mechanical or lack of mechanical stimulation from the arrest of respiration, sudden changes in blood pressure and pulse, brain anemia, secretions or other substances in the blood are not secondary factors for eliciting a bigeminal pulse in the insufflation experiments. If this is true, this arhythmia must be produced by a direct nerve impulse to the heart.

In the insufflation experiments there is no evidence that the carotid sinus functions as an afferent center for producing an arhythmia directly or indirectly.

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STUDIES IN THE PHYSIOLOGY OF EXERCISE

IV. Further Concerning Exercise and Basal Metabolism in Dogs

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Received for publication June 9, 1930

About two years ago, one of us (Steinhaus, 1928) reported on the influence of exercise on basal metabolism in dogs from observations made between May, 1925 and January, 1927. The present paper presents further observations on the same dogs for the period from January 1927 to December 1929. The literature which was reviewed in the earlier article showed a majority of workers supporting the view that exercise increased the basal metabolism. A much smaller number whose ranks we were led to join found exercise either having no effect on the basal metabolism or perhaps slightly reducing it. It is gratifying to us to note that of the workers who have written on the subject since then all have joined the ranks of what was then the minority. Lee and Van Buskirk (1928) observed the spontaneous activity of the rat in relation to basal metabolism in thyroidectomy, thyroid feeding, oestrus, and castration. They say: "The evidence is entirely against any idea that the amount of spontaneous activity manifested by an animal is dependent upon the basal metabolic level. Neither does the basal metabolic level show any close variation with the amount of spontaneous activity manifested." Benedict and Finn (1928) collected from the Nutrition Laboratory records the basal metabolism data of 19 different individuals who had been tested before and after a total of 29 summer vacation periods. The metabolism of 2 individuals was increased in a total of 3 vacation periods, but for all the others even though the vacation of at least a month provided considerable increase in muscular activity, they conclude: "The subjective impressions of individuals following a recuperation period in the Summer are so clear and indicate so frequently a pronounced change in physical and mental well-being, that it would seem almost as if during the Summer vacation individuals were undergoing a process of more than mild rejuvenation. . . . .The basal metabolism of an individual is in general sufficiently fixed as to be unaltered by a Summer vacation, even when pronounced subjective impressions of regeneration are experienced." Griffith et al (1929) conclude from their

studies on normal men and women that "vacations even when spent in such outdoor activity as a ten-day canoe trip with much tanning cannot be shown to affect the metabolism." Gemmill and co-workers (1930) from a study in muscular training report, "No significant changes were observed in the basal values of metabolism . . . ."

PURPOSE OF THE PRESENT INVESTIGATION. Since exercise makes demands on the fuel supplies of the body, the metabolic worker in this field finds himself alternating between two horns of a dilemma. If he exercises without increasing the intake, he is likely to have the equivalent of an undernourished animal on his hands while if he feeds more he faces the effects of increased food intake on metabolism, variously known as "luxus konsumtion" by Grafe (1923), "higher metabolic plane" by such as Benedict and Ritzman (1923) and by Wishart (1928) definitely related to the total urinary nitrogen. Both Grafe (1923) and Kleitman (1926) found rises in basal metabolism following increases in diet with or without more N.

In the face of such complications we found it necessary to observe animals while under three different regimes characterized as follows:

1. Exercise without addition to the diet.
2. Exercise with sufficient increase in food to hold body weight constant.
3. Exercise with dietary additions sufficient to permit an increase in body weight.

The results of the first program comprised the data of our 1928 paper. The present paper reports our experiences with the second and third regimes.

EXPERIMENTAL METHODS. Not only are the three dogs used in the tests here presented the same but the method of handling them, the standardization of laboratory and kennel routine, the control of activity and inactivity, sanitary conditions, etc., in fact all techniques employed were essentially the same as those fully described in the earlier paper. In addition we followed the plan of testing our apparatus for leakage during *every* metabolism determination. As already mentioned, the point of difference lies in the fact that additions were made to the diet. The control of the weight in accord with the requirements of programs two and three required a number of changes in the dietary allowance. All changes are shown in table 1. (See note at end of this paper.)

RESULTS. Our data are presented in table 2 (see note at end of this paper), in figures 1 and 2, and in the protocols of the three dogs. Table 2 reports the number of tests per week, weekly averages of body weights, Calories per hour, calculated Calories per square meter per 24 hours, and percentage deviation from averages of normal dogs for all tests conducted. Figure 1 portrays graphically that portion of the data showing our observations when enough food was added to keep body weight constant

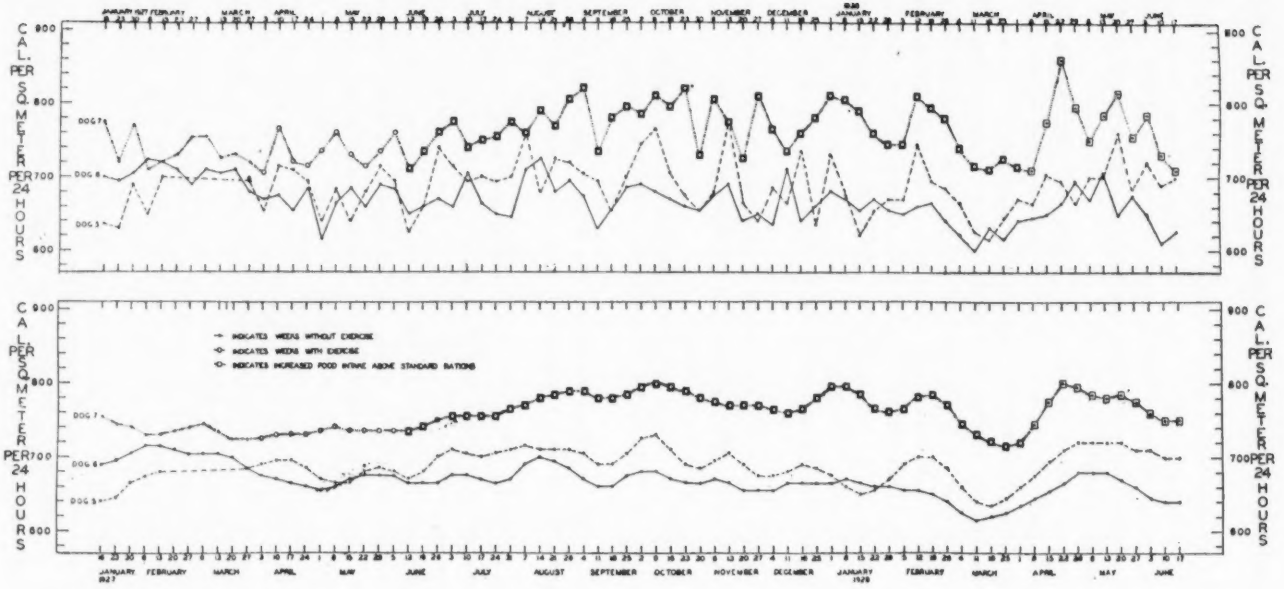

Fig. 1. Above: The averages of all metabolism tests for each week are plotted under date of the corresponding Sunday.
Below: The same data are plotted with curves twice smoothed by the method of running averages.

Dog 7 was run a total of 398 miles in the period from April 2, 1927 to April 2, 1928. Prior to this he had been unexercised for 101 weeks. The diet increase to prevent weight loss was begun June 12 and continued for 13 weeks beyond the exercise period.

Dogs 5 and 6 are unexercised controls.

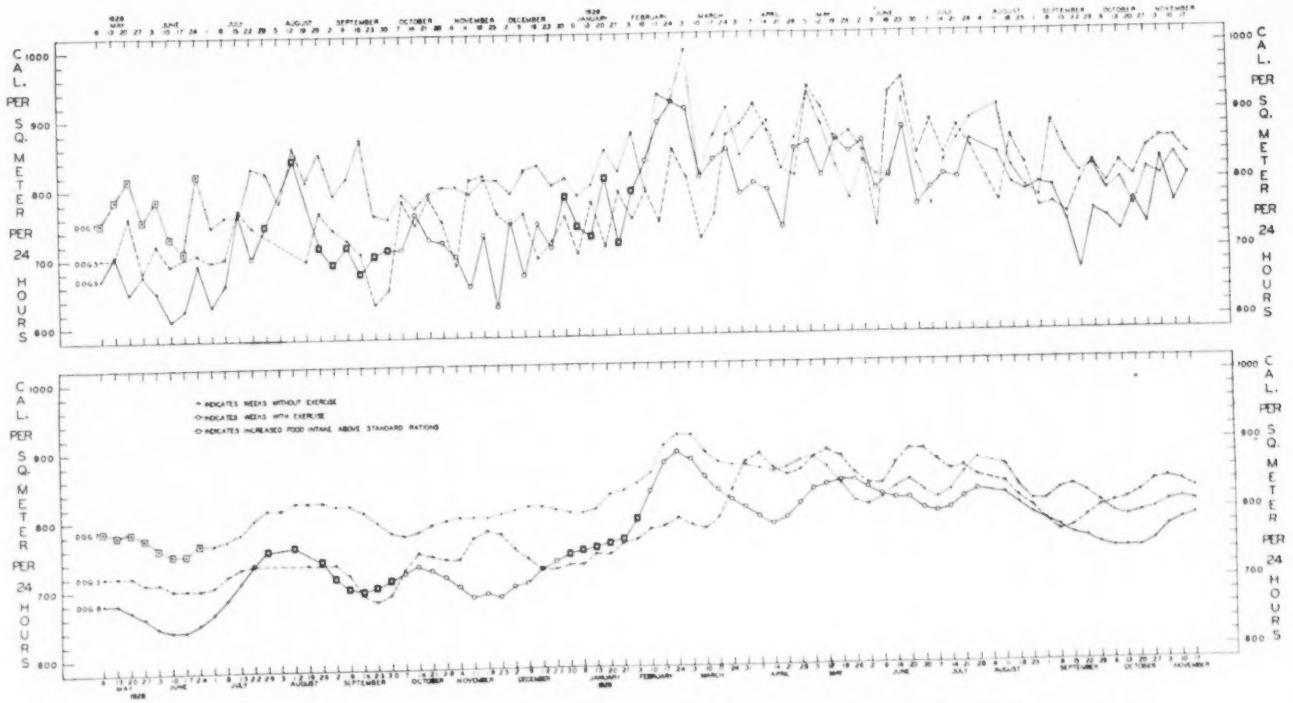

Fig. 2. Above: The averages of all metabolism tests for each week are plotted under date of the corresponding Sunday.

Below: The same data are plotted with curves twice smoothed by the method of running averages.

Dog 6 after 86 weeks of inactivity was run a total of 364½ miles and swam a total of 300 minutes in the period from August 1, 1928 to July 22, 1929. The diet was increased sufficient to permit a gain in weight from August 2 to October 11; the increase was in all elements of the diet whereas in the period from December 21 to February 8 the increase was in the protein fraction alone.

Dogs 5 and 7 are unexercised controls.

during exercise and figure 2 shows the results when enough food was added to permit increase in the weight of the exercised dog.

Protocol of individual dogs. Dog 5, a male mongrel, has been under continuous observation in our laboratory since March 24, 1925. His daily food ration has been both qualitatively and quantitatively unchanged throughout. This diet was described in detail in our earlier paper (Steinhaus, 1928). Since the summer of 1925, when he was exercised for ten weeks, he has been held as an unexercised control. To date (June, 1930) he has enjoyed 250 weeks of practical inactivity always indoors and confined to a small metal cage. Besides thrice daily crossing the kennel floor to empty himself, the only breaks in the monotony of his existence are the daily feeding and metabolism testing periods. Throughout the nearly three years reported here his weight has remained between 22.0 and 23.5 kgm. Excepting a two week period in February of 1927 when he suffered an infected burn, this dog has apparently been in the best of health. His nutritional index calculated by the Cowgill-Drabkin (1927) formula $\left(N_{obs.} = \frac{\text{Weight } \frac{1}{3}}{\text{Length}}\right)$ using 91 cm. as his body length has been about 0.31. These workers found that ratios of from 0.29 to 0.31 represented ideal nutritional conditions. Though always alert when shown attention he has learned to lie quietly in the bottom of his cage for the greater part of the day. In comparison with the exercised dogs, he is noticeably more clumsy and slow in movement. This dog had early shown comparatively low metabolisms vieing with dog 6 for lowest position. In the latter part of 1926 and early 1927, he frequently registered metabolisms as low as 620 Calories per square meter per 24 hours. In fact, his average of 610 for 6 tests in the week of November 21, 1926, we believe represents the lowest readings that have been recorded for normal dogs. In this light it becomes most interesting that in spite of the continuance of his inactivity throughout the present study, his metabolism has changed relative to the two other dogs so that for the last year and longer, he has shown basal metabolisms much higher than his own earlier readings and in fact for weeks at a time, he has been the highest among the three. There has been no noticeable change whatever paralleling this marked change in metabolism.

Dog 6 is our largest animal. His normal weight fluctuates in the neighborhood of 27 kgm. thus making his area when figured by the Meeh formula very close to one square meter. His Cowgill-Drabkin index at 27 kgm. and 92 cm. body length is 0.309. He also was caged on March 24, 1925 and in August was placed on his standard ration. He was used in the exercise series of 1925 and 1926 but had been unexercised for 86 weeks when in August of 1928 he started the training period designed to determine whether exercise would modify metabolism when enough food is added to permit an increase in body weight. In the period from August 2 to October

11, 1928 he was fed about 30 per cent above his standard ration. All elements of the diet were increased proportionately. This resulted in an increase in weight from 29.8 to 31.1 kgm. in spite of daily exercise. On October 12 he was returned to standard ration and his weight gradually returned to 29.9 kgm. On December 31, 1928 his food was again increased. This time however the addition was commercial casein, thus the increase was in the protein fraction alone. This represented an increase of 75 per cent in the N intake with but a 20 per cent calorie increase, and was continued six weeks, the weight fluctuating between 29.8 and 30.0 kgm. February 9 the diet was again reduced to standard but the exercise continued until July, 1929. The exercise was what we term mild but entirely sufficient to put the animal into excellent athletic condition. In the year of training, he ran a total of 364.5 miles at the rate of a mile in 12 minutes on a 22 per cent grade motor driven treadmill. During this year he also served in other experiments which required in total 300 minutes of swimming.

Dog 7, a bull terrier type mongrel, was caged May 21, 1925. He has always weighed between 20 and 22 kgm. At 20.2 kgm. his Cowgill-Drabkin nutritional index is 0.296. His body length is also 92 cm. By nature he is somewhat more excitable in contrast with the other two. Excepting a period of 4 weeks in the summer of 1926 when he ran a total of 12.5 miles, this dog had been unexercised for 101 weeks prior to April 2, 1927 when a year of training was begun. With him it was our purpose to add just enough food to hold the body weight constant throughout. He began the period weighing 21.6 kgm. Beginning June 13, a number of changes in diet were found necessary. These are indicated in table 1. On April 2, a year later when exercise was discontinued his weight was 21.8 kgm. For two weeks of the year his weight had reached a high mark of 22.1 kgm. Though a more than 20 per cent increase in dietary was maintained for an additional 13 weeks to June 30, his weight tended to fall. In the period from April of 1927 to April of 1928 he ran a total of 398 miles on our 22 per cent grade motor driven treadmill. He developed unusually excellent condition. Since then he has remained unexercised.

DISCUSSION OF RESULTS. Our 1928 paper supported the conclusion that "A period of training sufficient to put the musculature and the entire organism into excellent physical condition for strenuous work is without apparent influence on the basal metabolism either within the training period or in the subsequent post-training period." As mentioned, the dogs then reported were run without dietary increase and suffered moderate weight losses. In the present series, our dog 7 was exercised for a year without weight loss. His metabolism remained unchanged for the first ten weeks of exercise. Then in the week of June 12, the dietary increases began. From an inspection of figure 1, it appears that coincident with this increase, his curve draws slightly away from that of the controls. This

is particularly noticeable in comparing his with the curve of dog 6. This continues beyond the cessation of exercise early in April until June 1928 when his diet was again reduced to standard. A comparison with the other control, dog 5, shows similar though a little less marked trends.

Figure 2 represents our observations on dog 6 who was given enough food to permit gain in body weight. As already mentioned, food increases alternated with periods of standard rations. The first increase lasted eight weeks and permitted a gain of about 3 pounds (1.3 kgm.). His metabolism during this period shows no marked change relative to the other dogs. The second increase lasted six weeks, consisted entirely of protein and was paralleled by a distinct upward trend in the metabolism. The smoothed curve in figure 2 indicates that this drawing nearer to the control with higher metabolism did not abate until two weeks after the feeding of excess protein was discontinued. This increase appears to be definitely akin to the phenomenon reported by Wishart (1928) and is related directly to the extra protein consumed. Unfortunately for some unknown reason all of the dogs showed marked increases in their metabolisms beginning in the midst of this period of exercise for dog 6. Were it not for the fact that the exercised dog so consistently followed the curve of the unexercised dog 5 and held his position as lowest of the three except during the periods when extra food was added, this general disturbance of the basal metabolism might add difficulties to an interpretation of the data necessary to answer our question. From a consideration of all of the facts we are led to the conclusion that when pains are taken to distinguish between and evaluate separately the food and exercise effects, then it becomes evident *that exercise is without effect on the basal metabolism.* We do not believe that this position is inconsistent with one which maintains that exercise stimulates hypertrophy of skeletal muscles. It may however mean as Spiegel (1927) points out that muscle tonus plays a very insignificant part in the total metabolism. We have already said that the athlete somehow learns to relax very well. No doubt this is associated with a greater degree of "muscle awareness." In a personal communication, Dr. Edmund Jacobsen has said that he believes he has evidence from his long study of relaxation in man that in complete relaxation muscle tone is reduced to practically zero. Accepting this position makes it easier to harmonize exercise hypertrophy with unchanged basal metabolism. Schneider and Ring (1929) recently studied men in and out of training under basal, "resting" (sitting on bicycle ergometer), and work conditions. They say: "The resting metabolism, calculated for a square meter of body surface was definitely increased for both our subjects by physical training. . . . . It seems impossible to harmonize these data on the resting metabolism with our observations on the basal metabolism." Their data show reductions of 1 to 3 per cent in the *basal* metabolism in the training periods while

the same men in the same series showed increases of 2 to 12 per cent in the *resting* metabolism (sitting at rest on the bicycle ergometer) as the training progressed.

We are not unmindful that our dogs all show very marked variations in basal metabolism from day to day, from week to week, and from one period to the next. Unfortunately, we have as yet no explanation of this but we desire to make it very clear that most of our fluctuations rise and fall in terms *below* those reported by most workers with dogs. We suggest the following interpretation: If each of a dozen factors may independently modify the metabolism in a positive or negative direction the end result represented by the algebraic sum of all factors operating by chance at any one moment may be quite constant from time to time in spite of marked variations in the contributions of each factor. If now a considerable number of these factors are ruled out or held constant, then any trend in the remaining factors is much more likely to express itself completely and unrestrained in fluctuations of the total. It is under such conditions that one would expect lower metabolic rates and greater variations.

We have recently reported (Steinhaus, Jenkins and Lunn, 1930) that the average pulse rates of these dogs fall between 37 and 48. Rates as low as 28 per minute are not uncommon. In the fact that these figures are so far below those usually reported for dogs we find further support for the view that our dogs have attained a degree of relaxation greater than that of other workers.

SUMMARY

1. Basal metabolism data gathered in the latter three years of a five year study on three male dogs observed in and out of exercised states is presented and analyzed.

2. Dietary additions for the purpose of supporting the body weight during exercise were marked by increases in basal metabolism. This is in accord with the findings of others.

3. When care is taken to distinguish between effects due to exercise and those due to the increased food intake, the observations lend further evidence to the view that an extended period of training which puts the animal in excellent athletic condition is without noticeable effect on the basal metabolism per square meter per 24 hours.

4. It is proposed that the high degree of relaxation which is acquired by the trained animal may explain why it is possible to have muscle hypertrophy without an increased basal metabolic rate.

5. Marked spontaneous fluctuations around averages often much lower than those reported for normal dogs are noted. A position is developed which would explain the appearance of greater fluctuations coincident with

the attainment of more nearly true basal conditions. There is some indication that the fluctuations are cyclic in nature. Whatever their cause it is clear that any study of the effects of drugs, hormones or other factors on the basal metabolism must in some way reckon with these fluctuations. In the present work this was done: 1, by extending the observations over a long period of time; and 2, by holding at least one and usually two dogs as untreated controls to reflect the nature, trend, and extent of the spontaneous variations.

We are glad for this opportunity of acknowledging the invaluable counsel and inspiration of Prof. A. J. Carlson which we have enjoyed throughout the years devoted to this problem.

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Note: It has been found impractical to publish tables 1 and 2 with this paper. Reprints with the tables in full may be secured from the author or consulted in the following libraries where they have been deposited: Library of Congress and Surgeon General's Library, Washington, D. C.; Nutrition Laboratory, Boston; University of Chicago and John Crerar Library, Chicago; University of California, Berkeley; University of Toronto; British Museum; Bibleotheque Nationale, Paris; Staadts Bibliotek, Berlin; Gosudarstvennaya Publichnaya Biblioteka, Leningrad; Biblioteca del Academia Reale, Roma; Imperial University Library, Tokio; and Peking National University.

COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA

II. Synaptic Reactions in the Eye

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Received for publication June 16, 1930

Experiments indicating a marked interaction between separated regions of the retina have been reported in part I of this series (17). It was there shown that four test patches separated by an unstimulated area require a higher fusion frequency for flicker than when each patch is tested singly. It was also pointed out that this phenomenon is more prominent in the peripheral retina.

Adrian and Matthews (2) had previously found evidence of interaction between four such test patches in the eye of the Conger eel by employing the change in the latent period of discharge in the optic nerve as an index. The result of increasing the number of test patches in either of these experiments is similar to that produced by an increase in the intensity of the light illuminating a single area, a variation which also requires a higher fusion frequency or a shorter latent period. In comparing these two types of experiments, which it is assumed have a common explanation, it is interesting to note that the peripheral retina of the human eye and the entire retina of the Conger eel are structurally almost identical, both having a complicated network of lateral connections with receptors outnumbering the ganglion cells and a proportion between rods and cones of roughly 20:1. Such a structure of interconnected ganglion cells must be expected to favour interaction between neurones and the finding of Adrian and Matthews (2) that strychnine increases the range over which the phenomenon occurs gives striking evidence in favour of this view. The further conclusion that the interaction met with is of the nature of a spatial summation, a term understood to cover certain features of conduction in reflex arcs, has already been drawn (Adrian and Matthews, 2).

The work of Adrian and Matthews is particularly interesting from the point of view of physiological optics. It shows that among the facts found by physiologists and psychologists working on vision, some are dependent exclusively upon peripheral processes and should be explained along the lines suggested by these authors. By demonstrating the general

functional similarity of motor and sensory neurones they have made much of the work on reflexes applicable to vision in a way not indicated by the vague analogies hitherto suggested by various authors. On the other hand it should not be forgotten that the eye offers excellent facilities for studying quantitatively the general problem of conduction through synapses, having the advantage over most reflex work in the use of an adequate stimulus and an organ such as the retina whose structure is comparatively well known.

It has been noted by several observers, e.g., Charpentier (10) and Ives (22), that the fusion frequency for an intermittent light increases with the size of the area stimulated but so far as we are aware, this relationship between the fusion frequency and the size of the object has not been closely examined. The work on summation in the human eye has been carried out with methods which are all based on measurement of thresholds of intensity or colour for stimuli of different sizes. Now, as we shall see, there are aspects of summation which are not revealed by methods restricted to the feeble intensities necessary for evoking liminal values of sensations of light or colour. This limitation is overcome by using the flicker method which is adaptable to stimuli varying over a great range of intensities. This method also has the advantage of giving a comparatively small margin of error caused by subjective interpretation of the impressions and therefore shows satisfactory agreement between the results of experiments distributed over a long period of time. The method has also been put upon a more rational basis by virtue of the work of Adrian and Matthews which has served to elucidate the retinal basis of perception of flicker. On the excised eye they found the fusion point, defined by asynchronism of the discharge in the optic nerve, to increase with the size of the area excited, just as it did as the result of increased intensity of the stimulus. In line with this is the well known and often confirmed observation that the seen fusion point in experiments in vision is raised when the intensity of the stimulus is increased. At present therefore there is reason to assume that the eye perceives fusion when the discharging volleys of nerve impulses are asynchronous. (Cf. part I of this series, 17.)

The fusion frequency may therefore serve as a quantitative and objective measure of the intensity of the visual processes. In the present work we have studied the relationship between fusion frequency and intensity of stimulus, area of stimulus, and distance between stimulated regions of the retina. These determinations have been made for both the central and peripheral retina and have been used to test the hypothesis that the retina shows the same synaptic properties displayed by the central nervous system—interaction in general as well as the types of interaction known as summation, occlusion and facilitation.

METHOD. The light from a concentrated filament Mazda lamp was

projected on to a ground glass covered on the back by black paper with a hole for the beam and an adjustable diaphragm fitting close to the glass. The observer viewed the light patch through two eye holes in a dark screen in front of the glass at a distance of 50 cm. The light beam was interrupted by a rotating disc with two symmetrical opaque sectors of 90° each driven by a Singer sewing machine motor connected to the rotating axle of a Weston electric tachometer. By watching the voltmeter of the speed counter and compensating for any deviation of the needle, the speed of the motor was kept constant to within less than $\frac{1}{10}$ of a revolution per second, by means of a speed regulating rheostat in series with the driving motor. The tachometer showed an accuracy of less than 1 per cent. Two calibrations of it, the one by means of an ordinary speed counter and a speed reducing wheel system, the other one—three months later—by photographing a light beam interrupted by the rotating disc and by the lever of a Jacquet time marker, gave a difference of only 0.02 revol. per sec.

The illumination of the room as determined by the average size of the pupils of four observers placed before the dark screen was at about 0.032 millilambert. This value has been read from the graph published by Reeves (29) which shows the relation between the illumination and the average size of the pupils of 6 observers. After each observation experimenter and observer changed places. The former kept the disc running at a constant speed, unknown to the latter. Attention was paid to the necessity for fixating steadily even when, as in experiments on the peripheral portions of the retina, fixation time was no longer than one second.

The lamps were calibrated in a Lummer-Brodhun photometer against a standard furnished by the Bureau of Standards. The 110 volt D.C. supply operating these lamps was stabilized by shunting a set of condensers across the mains. In later work a 110 volt high capacity storage battery was available.

The intensities, given in meter candles, refer to the illumination projected on to the ground glass. Two lamps were used, of 144.0 and 63.2 candle power respectively. The highest intensity employed was obtained by moving the stronger lamp nearer to the ground glass; otherwise, the intensity was varied by means of neutral tint Wratten filters (nos. 1 to 3). The lowest intensity used was 0.094 meter candle (m.c.) The background was kept constant throughout the series at an intensity of 0.037 m.c. We have used the arithmetical means of three double readings taken on different days, the individual readings being controlled with speeds just over and under the fusion frequency.

THE RELATION BETWEEN AREA STIMULATED AND THE FUSION FREQUENCY AT DIFFERENT INTENSITIES. Experiments covering a large range of intensities have been carried out with two observers. In spite of considerable individual differences the findings have been constant and show an in-

creased fusion frequency with increased area. This increase is larger with high intensities and greater in the periphery than in the centre. Such typical results are well illustrated by figure 1 in which the fusion frequencies (one-half the number of flashes per second) are plotted as ordinates against log area as abscissa. The numerals against the curves represent identical intensities in the center and in the periphery, the values of which are given below the figure. At the lowest intensity stimulus and background are of nearly equal brightness when the disc is in rotation. At this low intensity

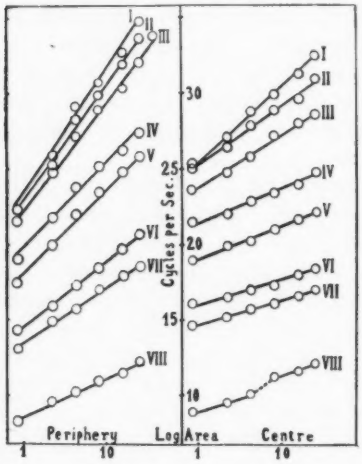

Fig. 1

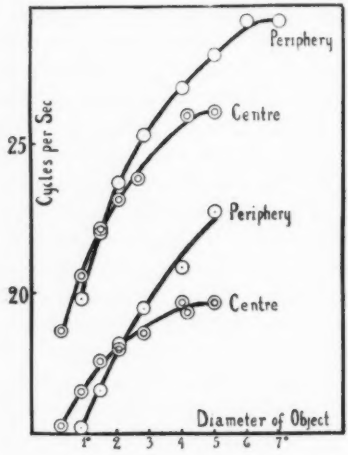

Fig. 2

Fig. 1. Subject R. G. Figures below abscissa give area in square degrees of diameter. Radius of circles marking observations drawn to correspond to average maximal deviation from the mean. Explanation in text. I = 428.6, II = 214.3, III = 94.0, IV = 21.43, V = 9.4, VI = 2.14, VII = 0.94, VIII = 0.094 m. c.

Fig. 2. Subject R. G. Six volt storage battery and Mazda headlight lamps. Upper curves: high intensity. Lower curves: 0.1 time this intensity. Absolute values not determined.

(Cf. curve *VIII*, fig. 1, central fixation) the heterogeneity of the receiving apparatus of the fovea and the paracentral area may have introduced complications. It is signficant that the break in the curve occurs at the visual angle with which the test patch transgresses the rod-free area. But for all intensities above this, both in the centre and 10° out toward the periphery there is a linear relation between fusion frequency and log area in accordance with the formula

(1) $$n = c \operatorname{Log} A + d$$

where n = fusion frequency, A = area and c and d are constants. The well known Ferry-Porter law (Ferry, 16, Porter, 27) similarly relates fusion frequency and intensity.

$$n = a \operatorname{Log} I + b \tag{2}$$

Although this is an approximation which sometimes shows deviations surpassing the limits of error (Lythgoe and Tansley, 24, and below, p. 221) it is often surprisingly accurate (Porter, 27; Ives, 22) and therefore seems to be too useful to be left without consideration as has been proposed.

In our formula (1) the constant c determining the slope of the curve increases with the intensity of the stimulus. This is true for both the centre and the periphery but a comparison of the curves in figure 1 for these two parts of the eye shows that the slope of the peripheral curves is influenced to a greater extent by the intensity. Thus at higher intensities the peripheral curves become steeper and steeper in comparison with the central ones obtained at corresponding intensities. The effect of size of area stimulated is therefore relatively greater in the periphery and at higher intensities.

It is to be emphasized that there is no absolute difference between the fusion frequencies of central and peripheral stimuli. This is evident from figure 2 which gives typical results for two observers in both the centre and periphery and shows that the size of the object is a determining factor: small objects give a lower fusion frequency in the periphery than in the centre whereas large objects give a lower value in the centre than in the periphery. The difference between the fusion frequencies in the centre and in the periphery is also determined by the brightness of the test patch. If the fusion frequency is higher in the periphery than in the centre the difference becomes still greater as the intensity of the stimulating light is increased. This is in accordance with the findings of Sherrington (31) and Lythgoe and Tansley (24).

The curves of figure 2, if plotted against log area instead of against diameter, would also show the linear relationship between area and fusion frequency illustrated in figure 1. From these data we may infer that the range of approximate validity of equation (1) involves a minimum of at least 0.09 sq. mm. of retinal area, corresponding to a diameter of 0.95 mm. or 21′ of visual angle and a maximum of about 2.66 sq. mm., corresponding to a retinal diameter of 1.63 mm. and a visual angle of 6°. Above that value the straight line will tend to become horizontal, as indicated by figure 2. Below the minimum a similar tendency must become evident at a certain size as there undoubtedly is a minimum fusion frequency above zero. Thus there is reason to believe that the complete curve relating the fusion frequency to log area is S-shaped and the equation (1) is an approximation referring to its rising branch, or perhaps only to the upper part of it. A similar curve is obtained when plotting fusion frequency against log

intensity as abscissa. Its lower part and ascending branch have been determined by Ferry (16), Porter (27), Ives (22), Lythgoe and Tansley (24); this with its upper, more horizontal, portion is plotted below (figs. 5–6).

Because of the variation of fusion frequency with the size, intensity and retinal location of the stimulus it becomes easy to see why the question as to the relative frequencies of fusion of an intermittent light in central and peripheral vision has given rise to so much controversy in the past (Cf., e.g., papers by Ives, 22; Allen, 7; Woog, 36, and others).

Figure 1 also shows that at maximal intensities the limiting value of the critical frequency is reached at different intensities with objects of different size—and regularly first with the small ones—and also at different intensities in the centre and in the periphery (cf. the curves *I*, *II*, and *III*). There the compared values of the fusion frequency of the centre and periphery will be determined by the limiting critical frequency obtained under different conditions. These questions will be reconsidered below (p. 221).

The high fusion frequency of the periphery at high intensities and with large objects is strongly indicative of a summative mode of function in this latter part of the retina. This was the explanation suggested in part I (17) to account for the difference found between central and peripheral vision. In view of the fact that the relation (1) holds both for central and peripheral vision and in view of the likewise common increase in the value of the constant C as the intensity increases, it would seem natural to assume this difference between the two parts of the retina to be quantitative rather than qualitative. But such an inference is difficult to reconcile with the facts derived from measurements of the visual acuity at different intensities, established by Uhthoff (34), Koenig (23), Roelofs and Zeeman (30) and others. The resolving power of the fovea increases with the strength of the illumination. How then would it be possible to separate adjacent stimuli at a small distance at high intensities if by virtue of the increased spatial summation they would tend to fuse?

SPATIAL EFFECTS AND THEIR INFLUENCE ON VISUAL ACUITY. In order to attack this important question experimentally the apparatus illustrated in figure 3 was designed to allow two bright half-circles to be set at different distances from one another. Their fusion frequencies were determined separately, S, and simultaneously, D. With central fixation (0°) the fixation point was in the middle between them. From this point to the peripheral fixation point the distance was 10° of visual angle. In the periphery only a few values for the single patches were sampled at random. In the centre a control of the singles was taken at nearly every distance tested, as regular variations in the fusion frequency were sometimes found, due no doubt to the greater heterogeneity of the central receptor pattern and its ganglionic connections.

The question was: would the fusion frequency in the centre for both patches together suddenly rise when they were just about to fuse into a complete circle, or would it rise gradually as the stimulated areas approached one another. The latter type of behaviour clearly was to be expected in the periphery on account of the experiments described in the previous paper (17).

In spite of individual differences the experiments show conclusively that the fusion frequency increases gradually as the half circles are brought closer together in both the centre and the periphery. Typical results,

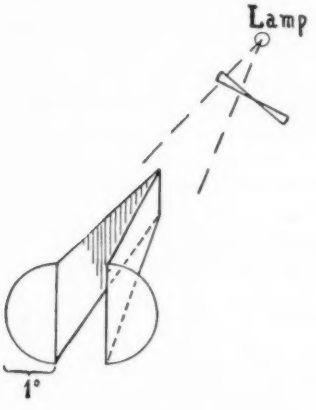

Fig. 3

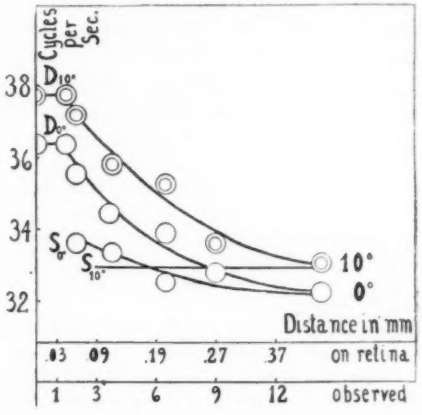

Fig. 4

Fig. 3. Scheme showing arrangement in experiment combining test on visual acuity with test on spatial effects. The angle between the opaque pieces is variable.

Fig. 4. Subject E. H. Abscissae: distance between semicircular test patches; $S_{0°}$ = single patches at 0°, $D_{0°}$ = both together at 0°, $S_{10°}$ = single patches 10° out towards periphery, $D_{10°}$ = both patches together at 10°. Intensity about 250 m.c.

except for the fact that the fusion frequency for the singles at 0° increases toward the foveola, are shown in figure 4 and indicate that the centre needs a somewhat shorter distance between the patches in order to show the same degree of interaction and that the central spatial effect is never as great as the peripheral one. At long distances (about 400 μ) only peripheral effects are found. The difference in range between centre and periphery is more marked at low intensities (cf. the previous communication, 17). The main outcome of this test, however, seems to be that the difference between the spatial effects of centre and periphery is merely quantitative.

Rather interesting is the fact that the central effects of interaction do not in the least interfere with the resolving power. With four observers the

spatial effect was practically complete at an intervening retinal distance of about 30μ between the areas stimulated, both in the centre and in the periphery, and yet the line separating them was seen as a perfectly steady, sharply contoured part of the background, about 1.5 mm. wide. It seems as if the interaction met with were confined to the areas stimulated, passing from one stimulated neurone to another and evoking little or no discharge in interjacent neurones. On this basis it is possible to see why spatial summation does not interfere with the resolving power. An additional possibility which must also be taken into account when synaptic reactions are concerned is that inhibition may occur at the edge of the area stimulated. Hering's (21) view, supported by some of Allen's (7) experiments, was that the mechanism of contrast, compensating for irradiation, enables us to distinguish sharp contours. Good spatial effects on the basis of the above experiment may be expected to favour visual discrimination. This conception is substantiated by the interesting observations of Wertheim (37) that

TABLE 1

*Lateral part of human eye**

RELATION	1 MM. NEAREST FOVEA MIDDLE	3.2 MM. PERIPHERAL	4.6 MM. PERIPHERAL	6 MM. PERIPHERAL
Outer nuclei / Ggl. optici	1.09	10.75	42.00	80.00

* The chrom-silver method of Golgi-Cajal that enabled differentiation between the cells with regard to their connections was not at that time (1889) in use, so that the outer nuclei need not all be the nuclei of rods and cones; the foveal value, however (1.09) indicates a considerable accuracy in Chiewitz's work.

visual acuity in the periphery increases with the number of elements stimulated. The low visual acuity of the peripheral retina demonstrated by Dor (14) and Wertheim (37) is probably less dependent therefore upon spatial summation than might at first be assumed.

The anatomical evidence agrees with the experimental results. The one to one relation between cones, bipolars, and ganglion cells in the fovea (Cajal, 9; Greeff, 18) does not exclude lateral connections, though the few rudimentary branches of the basal ends of these cones may diminish the number of possible junctions. The lateral connections are furnished by the horizontal cells and the cells of the ganglionic layer (Cajal, 9). Although their relative distribution in different parts of the retina does not seem to have been closely examined, we have not been able to find any evidence which would make probable the absence of lateral connections in the fovea. There are a great many amacrines around the fovea but they may have some specialized function since they are joined to incoming opticus fibres (Cajal, 9). Thus it is clear that the great foveal resolving power will have to be explained in

full recognition of existent possibilities of lateral spread of excitatory impulses. Cajal (9) did so when pointing out the one to one relation between the anatomical elements in the foveal receptor-path as opposed to the arrangement in the periphery, concerning which the above figures (table 1) given by Chiewitz (11) convey an idea.

The convergence of many receptors upon single ganglion cells will be partly responsible for the greater amount of summation in the periphery and almost certainly it is one of the main reasons for its low visual acuity. The retinal basis underlying the interaction must be furnished by the lateral junctions. On many grounds this conclusion is unavoidable (cf. Adrian and Matthews' work). The main difference between the centre and the periphery with regard to interaction seems to be that the peripheral neurones may be further apart than the central ones and yet be functionally interconnected, probably owing to the particularly long branches of the horizontal cells found by Cajal (9) in the peripheral mammalian eye. In explaining the greater spatial effect in the periphery, the greater number of lateral connections in this region will also have to be considered.

In another way this experiment with the two half-circles serves to elucidate the retinal basis of interaction. It seems to show that the explanation previously given to account for the interaction between distant areas can also account for the spatial effects observed with increasing areas. As the half-circles approached one another their simultaneously determined fusion frequency became equal to the value obtained with the whole circle. This fusion frequency could be reached when optical fusion of the two patches into one was still far from being imminent, which means that our curves of figure 1 could have been obtained by varying the total area illuminated by means of two half-circles some distance apart instead of with complete circles (cf. below, p. 223).

These experiments make it difficult to believe that the number of receptors as such should account for the results with increasing areas. Means of interaction must, as it were, be given the neurones by choosing suitable distances between them. It should also be considered that in the periphery (at 10°) the number of receptors must be very much the same as in the fovea in equal areas, if all rods and cones be counted, and much less if—as according to Hecht (19) seems proper—the many receptors per ganglion cell (table 1) be taken as one unit. If merely total number of receptors be the determining factor the periphery would not be able to reach a higher fusion frequency than the centre. But a greater number of neurones stimulated implies statistically greater means of interaction (cf. Sherrington, 32) and thus indirectly the total number of neurones will be decisive.

In still another way the final value of the fusion frequency may be indirectly determined by the total number of receptors present in a given area, e.g., with regard to differences in threshold (Hecht, 19). It is significant

that the visual acuity increases with the logarithm of the intensity as does the fusion frequency (Koenig, 23). To account for the large variation in visual acuity with illumination, one must suppose that as the intensity increases high threshold receptors become incorporated and a smaller value for the *minimum separabile* can thus be obtained (Hecht). A greater total number of high threshold receptors will be present in a larger area and such an eventuality might be reflected in determinations of the critical frequency. It is however not possible at this time to answer the question as to the relative part played by the receptors on the one hand and synaptic reactions on the other in the complex functions we are studying.

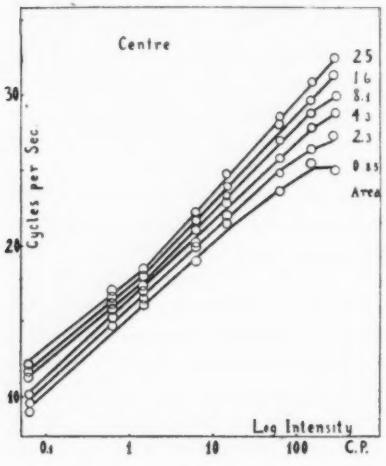

Fig. 5

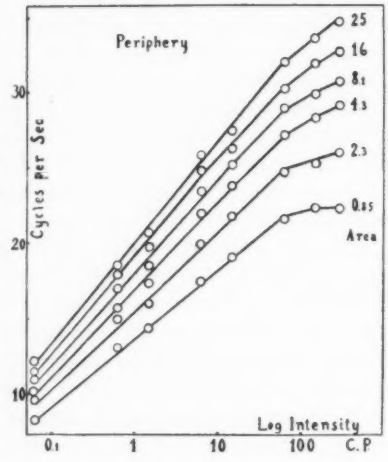

Fig. 6

Figs. 5–6. Subject R. G. Data of figure 1 p otted against log intensity as abscissa for objects of different size in centre (fig. 6) and periphery (fig. 7). Area is given in square degrees of diameter (see numerals against the curves).

THE RELATION BETWEEN INTENSITY OF STIMULUS AND FUSION FREQUENCY FOR OBJECTS OF DIFFERENT SIZE. The experimental data which were used in figure 1 to relate fusion frequency to log area are shown in figures 5 and 6 to represent the variation in fusion frequency with log intensity. Each curve presents data obtained over a period of about three months since any one experiment consisted in varying the area for a given intensity. Because the several curves thus represent an ensemble of experiments in which only one factor at a time was varied it is hardly to be expected that the Ferry-Porter law would be followed very accurately. And yet the figures show that for observer R. G. there is a linear relationship between fusion frequency and log intensity for objects of different size within a considerable

range of illuminations tested. The other observers gave a less satisfactory agreement with formula (2) but the data did not show a large deviation from the linear relationship and allow of conclusions similar to those to be drawn from the figures. The alteration in the slope of the curves for large areas in the centre at low intensities is to be ascribed to the heterogeneity between the rod-free fovea and the paracentral portions (cf. above, p. 214).

The outstanding results of these experiments are *1*, the increase in fusion frequency with intensity is greater the larger the size of the test patch; *2*, the upper limit of fusion frequency is higher with larger areas stimulated; hence a small object cannot be flickering at a rate maximal for a large object; *3*, the upper limit of critical frequency is reached at a lower intensity when the area stimulated is small. These statements hold for both central and peripheral vision. The centre, however, differs from the periphery in two ways; *4*, the difference between the maximal and minimal slope determining constant is smaller in the centre, i.e., the variation in fusion frequency with intensity is less dependent upon area in the centre,

TABLE 2

	α	β	γ	δ
Periphery	1.680	4.872	4.284	14.028
Centre	0.896	4.760	1.792	15.400

and *5*, the central curves reach the limiting fusion frequency at a higher intensity.

Most of these statements, as well as those referred to above (p. 214) in connection with figure 1, follow directly from the fundamental relations between area, intensity and fusion frequency summarized in the equations (1) and (2). Combining those equations one obtains

$$n = \alpha \operatorname{Log} I \operatorname{Log} A + \beta \operatorname{Log} I + \gamma \operatorname{Log} A + \delta \tag{3}$$

Where I = candle power (1.49 m.c.); A = square degrees of diameter; n = cycles per second. The relative values of the constants are shown in table 2. The various factors influencing the relation between fusion frequency and either area or intensity of stimulus can obviously be deduced from this equation. It is interesting to note that the two coefficients, α and γ, which undergo a significant change from the centre toward the periphery are those that enter the equation in the terms containing log area. The significance of statements *2* and *3* will be considered below (p. 224).

Before going further it is necessary to consider whether there is any reason for believing that an increase in fusion frequency due to spatial factors

need not imply an increase in the physiological process corresponding to intensity. Certainly at the present time there is little justification for making such an assumption and to do so would be going counter to the following four pertinent facts. 1. The formal identity of equations (1) and (2) referring to area and intensity respectively. 2. Accurate heterochromatic photometry is possible (Porter, 26; Ives, 22) on the basis of relating fusion frequency to brightness. 3. With liminal values a lower intensity may actually be used for the threshold if, within limits, the area stimulated be enlarged. And finally, 4, the general similarity between the results obtained by Adrian and Matthews with different criteria and those described above, both criteria pointing to increased intensity of the physiological process following an enlargement of the area stimulated. It thus seems necessary to conclude that any increase in fusion frequency is identical with a greater effective intensity.

A question of equal importance is whether there is any justification for assuming that the intensity of the physiological process is merely a function of the total number of neurones as such without regard to interaction. Undoubtedly such a view may be accepted since we know little about the mode of function characterizing the higher centres and it might be that they would interpret the discharge from a larger number of neurones as a more intense stimulation. The following four considerations however argue against this point of view and in favour of an interpretation based as largely as possible on synaptic retinal processes. 1. The evidence of section 4 (p. 216) showing that the spatial effects are due to interaction. 2. The main facts referring to spatial effects can be demonstrated on an excised eye (Adrian and Matthews, 1, 2, 3) in experiments which 3, further show that the fusion frequency defined by asynchronism of the impulses with regard to area and intensity behaves as does the seen fusion point in experiments on vision. A further point to be considered in this connection is that 4, the synaptic reactions which occur in the eye are to be expected on the basis of the anatomical evidence supplied by Cajal (9) who regards the retina as a "true nervous centre." It therefore seems wise to attempt an intepretation of these experiments on the basis of the conceptions developed by Sherrington and his co-workers for synaptic reactions. All the evidence indicates that the phenomena of interaction found in our experiments are of the nature of summation.

Nor is this view invalidated by the possible presence of receptors with a large range of variable thresholds. At least within limits an individual receptor is capable of discharging at a higher rate as the intensity of the stimulus increases (Adrian and Zotterman, 5) thus charging the more central retinal neurones at a higher frequency. Incorporation of receptors of higher threshold as the intensity increases will likewise charge these neurones at a higher rate both as a result of the increased number of receptors

contributing to the "frontal input" and the greater number of lateral junctions thereby brought into action (Adrian and Bronk, 4; Denny-Brown, 13). At present there seems to be no reason for distinguishing between these two sources of "input" to the sensory neurones: the decisive factor seems to be the total number of impulses impinging upon the neurones in unit time. On this view it is clear that the equivalence of area and intensity in equations (1) and (2) is not surprising but is rather to be expected. And likewise the greater spatial effects of the periphery are a consequence of the greater number of frontal and lateral paths converging upon the individual neurones.

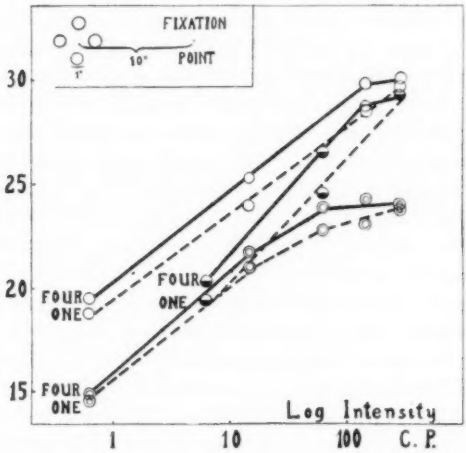

Fig. 7. Subjects P. H. ○, E. H. ◒, and R. G. double circle. Arrangement of experiment shown in upper left corner. Four = all patches simultaneously, one = each separately. Ordinates: cycles per sec.

Occlusion and facilitation. Convergence of a number of fibres upon a common neurone also presupposes what Cooper, Denny-Brown and Sherrington (12) have termed occlusion. If inhibition can be excluded the total effect will be determined by the balance of summation and occlusion. The latter term covers the case in which addition of fresh fibres impinging upon an already maximally discharging neurone is ineffective since the neurone is, so to speak, saturated. The presence of this phenomenon in the eye is demonstrated by the following experiment. Four test patches some distance apart as shown by figure 7 (upper left corner) are stimulated singly and simultaneously at different intensities. By choosing a sufficient distance between them we can satisfy ourselves that the effect of only a limited number of long lateral junctions in the case of the "fours" is com-

pared with the effect of a great number of frontal paths in a "single," plus a considerable number of always active "local" lateral paths within the "single." Figure 7 shows that introduction of more lateral paths raises the fusion frequency of the "fours" over the value obtained with the "singles" and the more so as the intensity increases. But at very high intensities the comparatively few long lateral paths are unable to add to the effect obtained with the frontal receptor-fibres and the short local lateral paths. The effect of the former is being occluded and the fusion frequency is now the same for both the "fours" and the "singles."

Evidently frontal as well as lateral effects may be occluded. Sherrington (33) has pointed out that the amount of overlap of excitatory paths in the central nervous system is measured by the deficit in the expected summation, the deficit being the occluded input. On this view the greatly overlapping peripheral neurones must become saturated at a lower actual intensity because of the greater input over both lateral and frontal paths and still be capable of giving a higher fusion frequency owing to the higher effective intensity as a result of the greater spatial effects. This, as we have seen, is actually the case (statement 5, p. 221). Thus an increase in the strength of stimulus above this point of saturation will be a "deficit" in the frontal input, which is larger in the periphery than in the centre for equal increments of intensity.

The presence of overlap and, consequently, of occlusion raises the question as to whether a field of neurones can be saturated by means of frontal or receptor-fibres alone. The supposition that the frontal input alone should be capable of charging the neurones maximally is extremely improbable since on this view the spatial effect would diminish at high intensities, whereas we have seen that it unquestionably increases with the intensity of the stimulus. Some other important facts are equally incompatible with the possibility of complete frontal saturation, whereas they are easily explained on the basis of the contrary assumption. Take, for instance, the striking result that with sufficiently small areas the periphery gives a lower fusion frequency than the centre. Owing to the great convergence of receptors upon ganglion cells in the periphery the number of lateral paths decreases rapidly with the size of the object and soon the peripheral effect will be determined more or less by the frontal "input," whereas the great number of ganglions in small central areas still covers a considerable range of short lateral connections adding to the frontal effect in the centre. On this basis there is likewise a simple and rational explanation of the fundamental fact (statement 2, p. 221) that small areas cannot flicker at the rate maximal for large areas. It was shown above that the area could be composed of two half circles some distance apart or, in other words, that the stimulated areas need not form a unit field. The latter fact must be taken to mean that the amount of lateral "input" is decisive. Thus with small areas and

a small lateral input the neurones are far from being saturated when the region of maximal intensities is being reached and cannot be saturated via frontal paths alone. Possibly the lateral input also is occluded, just as in the case with the four test patches where the number of horizontal connections was likewise limited. With large areas the lateral input is much greater and therefore does not get occluded or what occlusion there is takes place at a somewhat higher intensity and rather refers to the frontal input (statement 3, p. 221). The liminal intensity of the light seems to be determined by the balance of occlusion against summation. It should be remembered that the lateral input, though capable of raising the fusion frequency, does not increase the frontal input, whereas an increase in the frontal input also augments the lateral effects.

Occlusion and summation are two functional aspects characterizing a central structure with overlapping excitatory paths both of which seem to be demonstrable in the eye. Occlusion is merely lack of complete summation of maximal effects. But summation may also give more than the sum of several independent effects (facilitation) (12), owing to the fact that the individual paths may act upon a common fringe of neurones which either path alone is only able to excite subliminally. Stimulated together, the afferent nerves suddenly bring a common field of now supraliminally excited neurones into activity. In one case two individual excitatory afferents developed a tension of 50 grams and 0 gram respectively, whereas the reflex contraction when both were stimulated together, suddenly rose to 2,500 grams or 50 times the sum of the individual effects (Eccles and Granit, 15). It would seem that the greater variation of fusion frequency with increasing area in the periphery, i.e., greater value of γ in equation (3) indicates that we have evidence of facilitation in the present experiments. The nature of this evidence is perhaps better understood if we consider an extreme case and ask whether at any intensity a doubling of the area gives a greater increase in the fusion frequency than a doubling of the intensity. This does not happen in the centre, but a comparison of the slope of the two upper peripheral curves of figure 1 (log area) with the uppermost curve of figure 6 (log intensity) shows that the slope of the log area curves is much steeper than the slope of the log intensity curve for the largest area. The experiments on visual acuity in section 4, however, indicate that facilitation cannot in the eye be as marked as in certain types of reflexes.

Thus it is possible to demonstrate in the eye all the important aspects of synaptic reactions of the summative type. Even though it were shown that our assumption that every increase in fusion frequency is equivalent to an increase in the effective intensity does not contain the whole truth, it is a sufficiently close approximation to justify the main conclusions drawn in this paper. If there be a spatial effect on the fusion frequency that does not

imply an augmentation of the effective intensity, e.g., interaction as mere synchronization in contradistinction to interaction involving summation, this factor would interfere with our reasoning only with regard to facilitation and to the difference in liminal fusion frequency between small and large areas at maximal intensities.

CONCLUDING REMARKS. The results of our experiments and the general views developed in this paper suggest a number of important questions in the special field of physiological optics of which only a few can be indicated in the space available.

Parsons (25), considering peripheral daylight vision as contrasted with central vision, once remarked: "There is overwhelming proof, derived from peripheral luminosity curves, minimal field and minimal time luminosity curves, that peripheral vision behaves in exactly the same manner as central vision, but with diminished sensitivity. Greater stimuli are required to produce equivalent responses, but if the stimuli are sufficiently great, the differences disappear, including even qualitative differences, so that the fields of vision for colours extend to the extreme periphery (p. 177)." The great summative power of the periphery seems to us to afford the clue to a solution of this problem. If the stimuli are strong enough, the summation in the outer parts of the retina will compensate—or even overcompensate—for any lack of sensitivity. It has been long known and repeatedly confirmed (cf. references by Baird, 8) that the peripheral colour fields also can be greatly extended by increasing the areas (Hueck, 1840). This evidently is wholly in accordance with our findings as well as with the explanation given to account for them.

It is worth noting that these experiments clearly demonstrate the necessity for considering not only rods and cones as the anatomical basis for functional differences between central and peripheral vision. *A priori* all low intensity phenomena must not be ascribed to the rods and care must be exercised in drawing too general conclusions from experiments in which intensity is the only variable, since the fusion frequency is a complex function of both area and intensity with different constants and liminal values (cf. equation (3)). Much work remains to be done, particularly at low intensities. Before this has been accomplished the present results cannot advantageously be linked up to the many interesting data on area and intensity at the thresholds. Under these conditions it is possible that the relative number of receptors of different thresholds also introduces complications.

SUMMARY

Using the flicker method as a quantitative measure of the intensity of the visual process we have studied the influence of size of area stimulated, intensity of stimulus and distance between a number of test patches upon

the fusion frequency. Our experiments support the view that the retina demonstrates synaptic reactions of the summative type as is to be expected in an organ which not only contains receptors but is also a "true nervous centre."

1. The experiments indicate the approximate validity of the Ferry-Porter law:

$$n = a \operatorname{Log} I + b$$

2. It is shown that a similar relation holds for the influence of area on fusion frequency and that, accordingly, the fusion frequency n is a function of both these factors, of the form obtained by combining the formulae referring to area and intensity respectively (p. 221).

3. The variation of fusion frequency with both intensity and area is greater in the periphery than in the centre. This is more pronounced with regard to area, a fact which is shown to be in agreement with the anatomical differences between the synaptic structures in these two retinal areas.

4. The effect of interaction upon fusion frequency has been shown by the fact that several patches stimulated simultaneously give a higher fusion frequency than when each one is stimulated separately. This increases as the patches are brought closer together although the effect may be maximal when the retinal distance between them is as much as 30μ. The interaction met with is interpreted as summation.

5. Central and peripheral retinal areas show only quantitative differences in summative effects. In central vision full spatial effects may be present without impairing the visual acuity.

6. At sufficiently high intensities the fusion frequency for a single patch becomes equal to that for four simultaneously stimulated areas. This is interpreted as being due to occlusion.

7. Large areas give higher liminal values of both fusion frequency and intensity. The relative amount of summation and occlusion with different areas and intensities furnishes a simple and rational explanation of these phenomena.

We are indebted to Dr. D. W. Bronk for valuable suggestions and criticisms.

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COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA

III. Some Aspects of Local Adaptation

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Received for publication June 27, 1930

The greater adaptability of the peripheral retina is one of the most obvious differences between central and peripheral vision. It was first noted by Troxler in 1804 that images falling on the peripheral portion of the retina quickly faded and finally disappeared, an observation that has been repeatedly confirmed since (Aubert, 7; Holth, 19; Dunlap, 11, and others). One factor at work in this process must be the local adaptation, but the final disappearance of a peripheral object during fixation, *fixation blindness* (translated from the Norwegian term "stirreblindhed" introduced by Holth, 19) seems also to involve another process, as indicated by Dunlap's (11) experiments, as well as by some observations of our own. The disappearance of the image is remarkably sudden. If this decrease in excitability be studied by following the gradual fading of flicker as fixation progresses one finds a coarse flicker preceding the final momentary abolishment of the seen effect of stimulation. This is strongly reminiscent of phenomena of binocular rivalry or reflex rebound and the analogy is further stressed by later reappearances and disappearances of the object.

Our problem was to follow the course of local adaptation. An attempt to solve the same question was made in 1877 by von Kries (21) who used concentric sectored discs and an equality of brightness method. He thus showed that the adaptive effect increased with the strength of the stimulus (see below, p. 234) and that within the first three seconds local adaptation seemed to be particularly rapid. The quantitative value of these experiments is greatly reduced by the fact that *1*, the area stimulated was heterogeneous with regard to the distribution of receptor types (large object in central fixation); *2*, the outer comparison ring fell on an especially adaptable area, and may also have interacted (Allen, 6; Granit, 15) with the fatiguing area; *3* the room was in nearly absolute darkness, which by creating dark adaptation not only exaggerated the effect of *1* and *2* but also introduced the problems characteristic of that state of adaptation.[1]

[1] This was at a time (1877) when von Kries had not yet elaborated the conception of a functionally and structurally duplex retina. The work is not included among his collected papers on vision (22).

However, von Kries' experiments agree in a rough, qualitative way with those of Adrian and Matthews (1) who have found the frequency of discharge through the optic nerve of the Conger eel to decrease first rapidly and then more gradually during exposure to light. With this method Adrian and his successive collaborators (1–5) (8) in a series of important contributions to the general physiology of the special senses have also been able to describe similar reactions in other end organs.

The method employed in the present work was to measure the drop in fusion frequency of a flickering light during fixation and use this decrease as a criterion of the degree of adaptation. Because of the presence of fixation blindness in the periphery we were there restricted to short fixation periods and comparatively high intensities, it being known that under such conditions the decrease in excitability due to adaptation does not reach the low value necessary for the development of fixation blindness. The use of the flicker method also made it necessary to avoid even small movements of the eye and we soon found that it was difficult to obtain accurate results if the fixation time were extended beyond six seconds. However, within this time and with moderate or high intensities it seemed possible to follow the course of local adaptation with a considerable degree of accuracy. An advantage of the method is that no comparison light is needed and the relation between fusion frequency and intensity of stimulus can be determined experimentally. Furthermore, although the conclusions drawn from our experiments do not presuppose that the decrease in fusion frequency during continued stimulation actually follows the curve relating critical frequency to intensity, there is much evidence in favor of it (Granit and Harper, 16). Allen's (6) work on effects of "fatigue" is based essentially on the truth of this supposition. Our results are presented both in terms of computed intensities and in fusion frequencies as determined experimentally.

The experimental technique has been described in a previous communication (16) and the general conditions as regards state of adaptation, brightness of background, etc., are exactly the same as in that work. The time of exposure was measured by means of a stop watch, the shortest time being 1 second. Times shorter than 1 second need not be considered in connection with the questions to be discussed below, though experiments in progress have shown them to be of great interest from other points of view.

LOCAL ADAPTATION IN VARIOUS PARTS OF THE RETINA. The difference between the central and peripheral retina with regard to rate of local adaptation is shown by figure 1. With foveal fixation there is very little, if any, drop in critical frequency during the first six seconds. The diameter of the object in this case was 2° and it was therefore falling on a practically pure cone organ. The size of the peripheral objects was $2\frac{1}{2}°$.

At 2° toward the periphery, i.e., within the macula, there is a definite effect of local adaptation, (more marked with observer R. G. (on the right)) although the typically peripheral curve is not reached until at 4°. The approximate curve at 10° for observer R. G. is also given in terms of intensities (= intensity necessary for eliciting corresponding original fusion frequency, other conditions similar). Below similar curves are plotted on a greater number of data.

Interpreted in terms of the results of Adrian and his co-workers our curves indicate that the centre is of the slowly adapting type to which belongs for instance the muscle spindle, whereas the periphery is a rapidly adapting organ, though by no means so rapid as the receptors at the hair

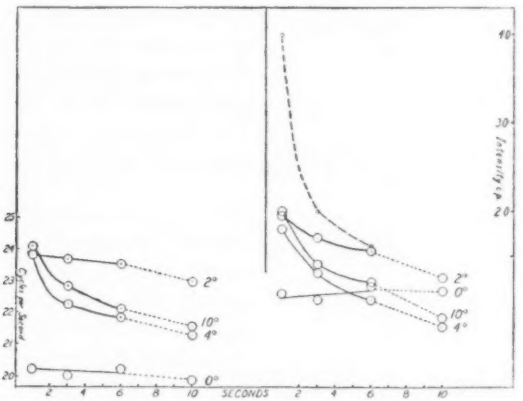

Fig. 1. Observers: v.A. (left), R.G. (right). The numerals against the curves refer to distance from fovea (= 0°) in degrees of visual angle. The dotted curve is the 10° curve of R.G. plotted against ordinates showing intensities computed from the critical frequencies.

roots which on Adrian's scale represent the other extreme. The validity of this interpretation rests upon the assumption that the main differences found refer to processes in the receptors. Since Adrian and Matthews (2), (3) obtained similar results in the excised eel's eye which greatly resembles the human periphery (15), we may safely infer that the phenomenon is essentially retinal. However, the peripheral and central retina differ not only as regards the relative distribution of rods and cones. An equally important difference between them is the amount of summative power (16) or, from the structural point of view, the amount of convergence of lateral and frontal (= receptor) paths upon the sensory neurones in the retina. It is, of course, possible that the many receptors converging upon the ganglion cells in the periphery (Chiewitz, 10; Cajal, 9) exert a

greater fatiguing effect upon those cells than does the single frontal path which in the fovea corresponds to each ganglion cell. However, in our opinion such an effect would not be so rapid as the one encountered. These questions will be considered experimentally in the next section.

ADAPTATION A RECEPTOR EFFECT. It was shown in parts I and II of this series (15), (16) that the convergence of lateral and frontal paths upon the sensory neurones leads to synaptic reactions such as occlusion and summation. The latter was described previously by Adrian and Matthews (3) as being present in the excised eye of the Conger eel. This being the case the total energy, as determined by the processes in the synaptic layers will be somewhat greater than the energy liberated in the neurone by each receptor. The total energy in the synaptic layers was shown to increase with the number of neurones activated owing to the increasing number of junctions. As a result of this the curve relating log intensity to fusion frequency has a steeper slope with larger areas. This is shown by figure 2, which is based on the results of the previous communication (16). Considering the points *A* and *B* it is evident that the intensity or energy per unit is the same. Because of spatial summation *C* reaches the fusion frequency of *A* in spite of its much lower energy value per receptor. It has been shown by von Kries (21) and his result will be confirmed below (p. 234) that, other factors being equal, the effect of adaptation increases with the intensity of stimulation as would be reasonable to expect. Accordingly, if the rate of adaptation were determined by total amount of energy, spreading over the synaptic layer, *A* should give a much larger effect than *B*, and *C* should also give an effect approaching that of *B*.

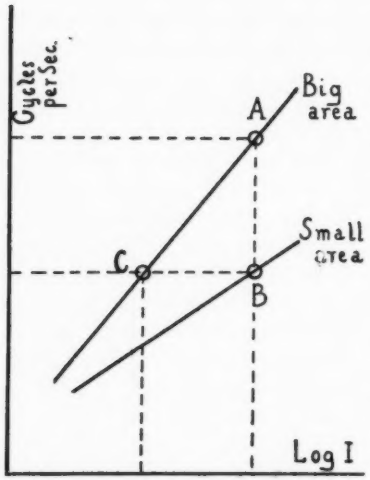

Fig. 2. Schematic. *A*, *B* and *C* are points selected to show equal fusion frequencies (*B*, *C*) for different area, and equal intensities (*A*, *B*) for different fusion frequencies on two curves relating log intensity to fusion frequency for two objects of different size. Data from the paper of Granit and Harper (16, fig. 7).

Table 1 shows the relations between stimuli *A*, *B* and *C* of figure 2. The intensities are calculated in relation to *A* (= *B*) which was at an intensity of 94 m.c. They are taken from smoothed curves relating log intensity to fusion frequency. However, as the differences between *A*, *B*

TABLE 1

TIME	R. G.			W. v. A.		
	Fusion frequency cycles per second	Calculated intensity	Per cent decrease	Fusion frequency cycles per second	Calculated intensity	Per cent decrease
	A = 4° diameter, high intensity					
seconds						
1	10.8 (0)	94.0	0	10.3 (0)	94.0	0
2	10.0 (0)	37.5	60.0	9.96 (0.053)	64.8	31.1
3	9.74 (0.046)	28.4	69.8	9.83 (0.056)	56.7	39.7
4	9.45 (0.03)	20.8	77.9	9.71 (0.05)	49.9	46.9
6	9.08 (0.056)	13.9	85.2	9.66 (0.05)	47.2	49.8
	B = 1° diameter, high intensity					
1	8.33 (0.04)	94.0	0	7.7 (0.03)	94.0	0
2	7.53 (0.11)	33.2	64.7	7.03 (0.09)	33.8	64.1
3	7.07 (0.09)	18.0	80.85	6.75 (0.10)	21.8	76.8
4	6.7 (0.067)	11.0	88.3	6.53 (0.056)	15.5	83.5
6	6.53 (0.04)	8.9	90.54	6.4 (0)	12.4	86.8
	C = 4° diameter, low intensity					
1	8.37 (0.04)	6.28	0	7.77 (0.03)	6.0	0
2	7.87 (0.04)	3.84	38.86	7.45 (0.03)	4.55	24.
3	7.52 (0.02)	2.59	58.76	7.33 (0.02)	4.02	33.0
4	7.3 (0.067)	2.08	66.9	7.22 (0.02)	3.57	40.5
6	7.12 (0.03)	1.71	72.8	7.12 (0.02)	3.23	46.17

and C with respect to area and intensity were considerable the main findings were not interfered with by casual variations. As mentioned above a direct comparison of the fusion frequencies (table 1) will show the important points brought out by this experiment. Since in our opinion it is safe enough to compute the effective intensity from the critical frequencies, we prefer to discuss the results as they appear in terms of percentage decrease of brightness during continued fixation (fig. 3 and table 1). A comparison of stimuli A and C which only differ in intensity confirms von

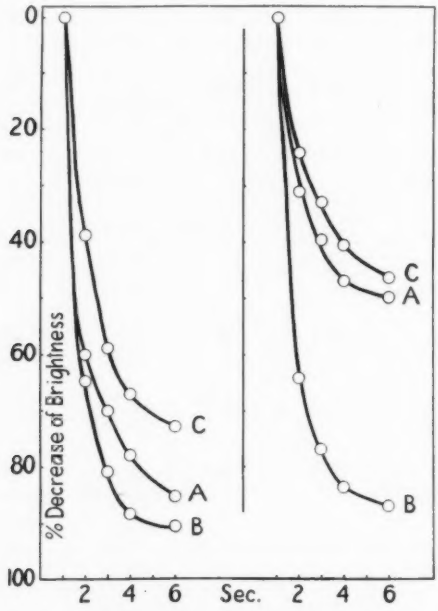

Fig. 3. Observers: R.G. (left) and v.A. (right). Region of stimulation 10° toward periphery. The letters A, B and C against the curves refer to conditions of area and intensity as indicated by the same letters in figure 2 and table 1.

Kries' observation that, other factors being equal, the stronger stimulus gives the greater effect. As to the main issue, figure 3 and table 1 clearly show that an increase in the total amount of energy does not in the least accelerate the rate of adaptation, since the decrease of A actually is smaller than that of B. Likewise C gives a much smaller drop in intensity during fixation than B. Thus there is no spatial compensation for lack of intensity in C as far as adaptation is concerned. As the process of adaptation is not accelerated by an increase in total energy propagated along lateral

paths of interaction later in the synaptic layers it must be "presynaptic," evidently determined by the nature of the receptors. This outcome of the experiments was to be expected on account of the results of Adrian and his co-workers. They found adaptation to be a general property of the sense organs, not confined to receptors which like the eel's eye have a central structure interposed between the actual end-organ and the afferent nerve.

The fact that *A* did not give a greater drop in excitability during fixation than *B* thus justifies the conclusion that total energy, as defined by the particular properties of synaptic structures, does not primarily determine the rate of adaptation. But it is nevertheless probable that the neurones may be capable of modifying the final response. The fusion frequency in the end is influenced by synaptic processes as well. Inasmuch as the central structure is more efficiently charged the larger the area stimulated, the "central excitatory state" (Eccles and Sherrington, 12) in this case drops less rapidly to subliminal values (Sherrington, 26) and may thereby retard the appearance of the maximal adaptive effect. In other words, the excitatory state in the neurones of the small area is more dependent upon the frontal (receptor) input. In the large area the greater lateral input compensates for loss in frontal input through adaptation. Thus we may understand why the drop with a large area, *A*, is even smaller than the drop with a small area, *B*. Although other interpretations of this phenomenon may be suggested this one seems best to fit the experimental facts at present.

Finally it is worth noting that these experiments are of some general interest as a type of approach which makes it possible partially to localize processes in the eye by using the effect of the size of area stimulated as an indicator. In this particular case the local adaptation has been found to take place in the end organs and further experiments have been planned in which objects of *constant* size will be compared as to other variables with the aim of obtaining data enabling a closer understanding of the processes in the photoreceptors.

INFLUENCE OF RED AND BLUE LIGHTS ON LOCAL ADAPTATION. The conclusion that the rate of local adaptation is determined by the nature of the end organ will be subjected to another test in this section. At the same time we will try to gain some information regarding an important—and hitherto much neglected—question referring to the concept of a functionally and structurally duplex retina, developed by Schultze (1868), Parinaud (1881) and in particular by von Kries (1894 (22)).

There seems to be no doubt about the general validity of this concept in so far as it presupposes a close correspondence between rods on the one hand and scotopic vision on the other and more and more cones becoming active as the photopic state of adaptation is approached. But as yet it is

an open question as to how the rods function in daylight vision (cf. Parsons, 23, p. 177). Some writers (cf. e.g., Piéron, 24) assume that red light to which the dark adapted eye undoubtedly is less sensitive (e.g., Purkinje effect) does not stimulate the rods under any conditions. Grindley (18) furthermore has recently gone so far as to apply a crucial test to a hypothesis concerning interaction between rods and cones (Granit, 13, 14) on the basis of this rather hypothetical assumption. It should be remembered, that experiments in scotopic vision do not give any information as to the function of the rods in photopic vision, that there is no reason to believe that rods saturated with visual purple react like ones that have

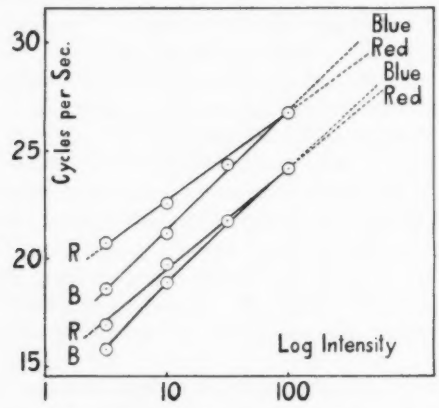

Fig. 4. Observers: R.G., upper pair of curves, v.A., lower pair. Region of stimulation 10° toward periphery. Diameter of object 3° of visual angle. Fusion frequency—log intensity (arbitrary units) curves for red and blue light.

had their visual purple bleached, and that, furthermore, phenomena at the absolute threshold showing selective sensitivity to the one or the other wave length hold only for the receptors of lowest threshold. It is also questionable whether absolutely valid conclusions as to general differences between rods and cones can be based upon differences pertaining to central and peripheral daylight vision. The fovea is a highly specialized organ, differing somewhat from the periphery as to types of cones[2] but especially with regard to synaptic arrangements (Greeff, 17) which in parts I and II

[2] To prevent misunderstanding it should be mentioned that with this remark we do not intend to confess adherence to a line of reasoning which hypothecates functional similarity between certain types of rods and cones because of certain superficial structural resemblances between them. According to Greeff ((17) p. 123) it is always possible to distinguish a cone from a rod, the basal ends and the nuclei of the cones being characteristically similar all over the retina.

of this series (15), (16) were shown to cause specific functional reactions varying in amount with the distance from the fovea. The fact that in some respects the general concept of a functionally and structurally duplex retina is sufficiently well founded to be accepted should not be allowed to hide some of the questionable issues of the duplicity *theory* and its need of further elaboration.

Before describing the effects on local adaptation that were obtained with red (Wratten filter no. 27) and blue (no. 47) light let us consider the curves relating critical frequency to the logarithm of the intensity. Figure 4 shows them for two observers over the range in which we are interested. It will be seen that the slope of the curves referring to red and blue light respectively differ in a similar way for both observers. This is in agreement with Ives' (20) results. Two pairs of points on these curves were chosen for each observer and their intensity so adjusted that the red and the blue gave approximately equal fusion frequencies both for the upper high intensity pair of points and for the lower intensity pair. Table 3 shows that when the intensity of the upper pair is taken arbitrarily as 100, the red and the blue are of equal brightness (= equal fusion frequency) if the ratio of intensity for blue and red of the lower pair is 19.1:11.6 for R. G., 15.48:12.31 for v.A. Although the absolute energy values were not determined it is questionable whether they would aid materially in our reasoning, since the physiological effectiveness must depend upon the thresholds of the receptors and not merely upon the energy. A determination of the latter therefore need not give any information about the former, except in the particular case when physiological threshold values are compared with the amount of radiant energy needed for eliciting liminal effects. The important factor is the variation in the relative energies of blue and red at the two intensities that give equal brightness. This is shown by the slope of the curves and also by the following values (table 2) calculated from the known transmission of the coloured filters, interposed neutral filters (Wratten) and the intensity of the lamps. Therefore in

TABLE 2

	R.G.	BLUE/RED	W. v. A.	BLUE/RED
Blue—low intensity	15.6	1.43	7.64	0.70
Red—low intensity	10.92		10.92	
Blue—high intensity	81.1	0.86	49.3	0.56
Red—high intensity	94.1		88.7	

order to produce equal apparent brightness (Porter, 25; Ives, 20) at high intensities the red light requires relatively more energy than the blue as

compared with the ratio at the low intensities. Or on the other hand at a high intensity, there is relatively less energy of a short wave-length needed for giving an effect equalling that of a long wave-length than at a low intensity, provided that the range of the Purkinje effect with concomitant specific low intensity effects is not included.

Proceeding now to a comparison of the effects of adaptation with the red and blue lights used in the experiments of figure 5 the question evidently is whether the relative fall in intensity is determined by the factors that are equal, i.e., brightness, or by those that alter, i.e., energy values. The curves in figure 5 are plotted to show decrease in intensity directly instead of percentage decrease, although the latter values may be found in table 3. It can be seen in this table that the relative effectiveness of red and blue at the two intensities chosen alters just as the energy values. Taking for instance the percentage decrease in six seconds for observer R. G. the relation blue:red is 71.1:85.8 at the high intensity, and 64.26:60.35 at the low intensity to be compared with corresponding variations in energy of 81.1:94.1 and 15.6:10.92 respectively. This means that the red light gives a relatively greater adaptive effect than the blue at the higher intensity compared with the effects at the lower intensity. The energy variation shows, accordingly, that the light which requires relatively more energy for an equal increase in brightness has a greater effect on adaptation, which in this case was equal to lower final value in 6 seconds. This result wholly confirms our conclusion that the rate of adaptation is determined by energy per unit receptor. Thus von Kries' finding that higher intensities give more rapid adaptation is again confirmed. Although this confirmation follows from a consideration of the percentage decrease in brightness it is especially striking if the actual drops in intensity are compared.

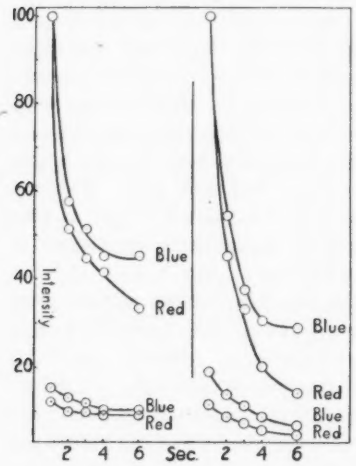

Fig. 5. Observers: v.A (left), R.G. (right). Course of local adaptation for red and blue light at two intensities (see table 3). Ordinates computed from the data of figure 4 (see text).

Figure 5 also shows that if the difference in intensity between the two blue lights or between the two red lights is large enough, the high intensity

curves tend to flatten out before the lowest value of the low intensity curves is reached. This may be accounted for by a compensation for loss in frontal input through lateral channels (as above with regard to area, p. 235) or by differences in threshold.

TABLE 3

SECONDS	RED						BLUE					
	R.G.			W. v. A.			R.G.			W. v. A.		
	Fusion frequency	Calculated intensity	Per cent decrease	Fusion frequency	Calculated intensity	Per cent decrease	Fusion frequency	Calculated intensity	Per cent decrease	Fusion frequency	Calculated intensity	Per cent decrease
					High intensity							
1	26.77 (0.13)	100	0	24.12 (0.13)	100	0	26.74 (0.09)	100	0	24.15 (0.06)	100	0
2	25.37 (0.13)	45.2	54.8	22.82 (0.18)	51.29	48.71	25.37 (0.13)	54.5	45.5	22.96 (0.09)	57.6	42.4
3	24.53 (0.15)	33.2	66.8	22.54 (0.18)	44.67	55.33	24.44 (0.11)	37.6	62.4	22.74 (0.16)	51.29	48.71
4	23.94 (0.09)	20.2	79.8	22.40 (0.18)	41.69	58.31	24.00 (0.28)	30.6	69.4	22.40 (0.28)	45.19	54.81
6	23.30 (0.40)	14.2	85.8	22.12 (0.37)	36.31	63.69	23.86 (0.28)	28.9	71.1	22.40 (0.28)	45.19	54.81
					Low intensity							
1	22.96 (0)	11.6	0	19.94 (0.06)	12.31	0	22.82 (0.18)	19.1	0	19.94 (0.06)	15.48	0
2	22.48 (0.12)	8.8	24.06	19.49 (0.13)	10.00	18.77	22.12 (0.18)	13.9	27.2	19.52 (0.12)	13.18	14.86
3	22.12 (0.09)	7.25	37.5	19.46 (0.09)	9.89	19.65	21.36 (0.26)	11.3	40.84	19.32 (0.18)	12.03	22.29
4	21.70 (0.28)	5.76	50.35	19.32 (0)	9.12	25.92	21.06 (0.11)	8.8	53.93	18.98 (0.06)	10.48	32.30
6	21.31 (0.23)	4.6	60.35	19.32 (0.09)	9.12	25.92	20.44 (0.28)	6.83	64.26	19.98 (0.21)	10.48	32.30

None of the facts revealed by a detailed analysis of the relation between adaptive effects and energy values are so striking as the general similarity of the reactions to red and blue light. This is shown particularly in figure 5.

The type of behaviour is the same in the two cases, and is in marked contrast with the adaptation in the rod free fovea. We must conclude that the peripheral rods and cones are either functionally very similar or that both wave-lengths stimulate both types of receptors, since we know that the first rapid drop in effective intensity—or fusion frequency—is caused by a decrease in excitability in the *end organ*. It seems to us that if the former suggestion be accepted then there is little left of the duplicity theory except the obvious difference between the fovea and the rest of the retina. The fundamentally different general adaptability of the two types of receptors (dark adaptation) is one of the main facts underlying the concept of a functional duplicity related to rods and cones. Why then should they behave so similarly with regard to local adaptation?

On the other hand the assumption that above a certain intensity both red and blue lights stimulate both types of receptors is more in accordance with the duplicity concept. The first rapid drop which is so typical a characteristic of both the human peripheral retina and the eel's retina must then be a rod phenomenon. This is stressed by the fact that the rapid decrease in excitability is not very marked until the edge of the macula has been reached (see above, p. 231). Finally, on the basis of the evidence of Adrian and his collaborators, there is every reason to correlate the marked differences in rate of adaptation with structural differences; the more so as in this case we have been able to show that the difference found between the fovea and the periphery is not dependent upon synaptic reactions. Thus the duplicity theory, if not too dogmatically interpreted, instead of being invalidated is actually supported by our observations and receives further development.

SUMMARY

The rate of local adaptation has been determined by following the drop in fusion frequency of an intermittent light during 6 seconds.

With central fixation there is little or no local adaptation demonstrable within the period in question. Passing towards the periphery the effect becomes more marked, and from 4° outwards there is a comparatively large drop in critical frequency during the first three seconds of fixation. The effect gradually diminishes in amount as the time of stimulation is lengthened.

The rate of local adaptation is greater with stimuli of higher intensity.

It has been found that the rate of local adaptation is determined primarily by the energy per receptor unit rather than by total energy increased by processes of summation in the synaptic layers of the retina. This was likewise shown to be the case in experiments with red and blue light.

A greater total amount of energy, however, seemed capable of causing a retardation of the rate of adaptation assumed to be due to more effective charging of the central structures in the retina.

In general the effect of red and blue light upon the rate of adaptation was essentially similar. This indicates that with intensities above the range of the Purkinje effect red and blue light must either stimulate both rods and cones or that there is practically no functional difference between peripheral rods and cones with regard to local adaptation. Neither assumption is in keeping with the concept of a complete functional-structural duplicity of rods and cones.

It is shown that the former assumption is preferable to the latter particularly on account of its greater conformity to the main facts underlying the duplicity theory. The first rapid decrease in fusion frequency with peripheral fixation is assumed to be a rod effect.

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SEX AND SEASONAL VARIATIONS IN EXCITABILITY OF THE CARDIO-INHIBITORY MECHANISM OF FROGS AND TOADS

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Received for publication June 30, 1930

Stimulation of the vago-sympathetic nerve to the heart necessarily involves two mechanisms, the inhibitory and the accelerator, and usually results in the predominance of the former. Exceptions to this rule have been claimed by many workers. Carlson (1) states that the inhibitory nerve fibers in Limulus did not fail to produce cardiac inhibition, as is sometimes the case in amphibians and mammals. Brinkman and van Dam (2) claim that in some frogs the cardiac vagus action predominates over that of the sympathetic nerves, in others the action of the sympathetic nerves predominates. Loewi (3) reports that in frogs during the winter season the threshold of excitation of the accelerator nerves is higher than that of the vagi. Cori (4), contrary to the description of Loewi, maintains that stimulation of the vagus in summer frogs does not cause cardiac inhibition. In such frogs paralyzing the sympathetic nerves with ergotamine or physostigmine cardiac inhibition is produced by the vago-sympathetic stimulation. Cori also reports that the change from the winter response to the summer response takes place at the mating season. According to Cori the variations in response might be due to variations in thyroid activity, since crude thyroid extract applied directly to the heart is said to abolish the cardio-inhibitory response to vago-sympathetic stimulation. Walderstrom (5), working with thyroxin, claims that thyroxin had no effect on the parasympathetic innervation of the frog's heart.

Barbour and Kleiner (6) were able to decrease markedly the response of the heart to cardiac stimulation of the vagus by means of adrenalin and caffeine. Heymans and Maigre (7) report that the injection of 0.4 cc. of 1 per cent solution of methylene blue into the lymph sac of frogs abolishes the cardiac inhibition previously produced by vago-sympathetic stimulation of a given strength. Haberlandt (8) showed that the frog heart restored to rhythm by irrigation with blood, after strychnine, caffeine, veratrine and quinine paralysis, is refractory to vagal inhibition. The same dissociation of the vago-cardiac mechanism was obtained after revival

from water rigor or heat rigor or freezing by chlorethyl. Asher (9) states that Ringer's solution, which has been repeatedly passed through the liver augments the heart beat and lowers the excitability of the vagus nerve to the heart. Burglia (10) reports that in the newborn cat, stimulation of the vagus causes slowing of the heart beat, but complete inhibition is produced only after five weeks of extra-uterine life. In the dog the latter effects are obtained only a few days after birth. Howell (11) states that the frog heart in potassium free Ringer's solution shows no cardiac inhibition on stimulation of the vago-sympathetic nerve. When potassium is added vago-cardiac inhibition is restored. Ten Cate (12) reports similar results, and Colle (13) claims that lessening of potassium in the perfusion fluid necessitates the use of higher minimal voltage for vagus excitation. An increase in potassium diminishes the required voltage. Busquet and Pachon (14) reported that the addition of calcium chloride to a potassium free Ringer's solution restored the cardio-inhibitory mechanism in frogs. Hagan and Ormond (15) claim that calcium is absolutely necessary for maintaining the cardio-inhibitory mechanism, but that potassium may replace a part of it. However, Brine (16) could not confirm the findings of the previous investigators. In her experiments the potassium and calcium contents were varied and in all cases cardiac inhibition occurred on vagal stimulation. Similarly, Cori states that he obtained cardiac inhibition in frogs on stimulation of the vago-sympathetic nerves, in potassium free Ringer's solution. Bonsdoroff and Granit (17) recently reported that rats fed on a diet deficient in fat soluble vitamins showed an increased excitability of the vago-cardiac mechanism.

METHODS AND RESULTS. Toads and frogs were employed in this work.

Toads. Fifty-eight toads were subjected to a systematic study of the effects of vagal stimulation on the heart. This work was done in Beirut, Syria, during the year 1928.

The experiments were performed with the hearts "in situ" and tracings obtained by means of a light heart lever. Body weights, heart weights, temperature and the barometric pressures were noted. The vago-sympathetic nerves were stimulated with strong and weak faradic currents.

The records obtained show that the effectiveness of the vagi nerves on the heart varies, in part, with sex. More marked inhibition is obtained in the female toads (table 1).

Frogs. A similar investigation was undertaken on frogs, in Chicago, during the months of August and October, 1929. Forty-two frogs were used with the following results (table 2).

Almost all the female frogs exhibited marked cardio-inhibitory effects on stimulation of the vago-sympathetic nerves, while in a large percentage of the male frogs the heart was vagus refractory. This sex difference, however, could not be obtained during the winter season (November-May), when marked vago-cardiac inhibition was seen also in males.

Further experiments were carried out in an attempt to analyze the underlying mechanism of the variations in response to stimulation of the vago-sympathetic nerves in frogs.

The hearts of the frogs which had proven to be inhibited by stimulation of the vago-sympathetic nerves were finely divided and ground in Ringer solution. The extract thus prepared was poured over the vagus refractory heart. After this application stimulation of the vagi produced cardiac inhibition. The sensitizing appeared in ½ to 1½ minutes and lasted for a period of 10 to 30 minutes, during which vago-sympathetic stimulations brought about inhibitory effects of gradually decreasing magnitude. This sensitizing could be repeated by further applications of the extract.

Similar extracts were prepared from various organs of the body of the frogs which responded by cardiac inhibition on stimulation of the vago-

TABLE 1

Toads

DEGREE OF INHIBITION	NUMBER OF MALES	PER CENT	NUMBER OF FEMALES	PER CENT
Complete inhibition	8	26	11	55
Partial inhibition	10	21	5	25
No inhibition	20	52	4	20

TABLE 2

Frogs

DEGREE OF INHIBITION	NUMBER OF MALES	PER CENT	NUMBER OF FEMALES	PER CENT
Complete inhibition	7	35	21	95
Partial inhibition	4	20	0	0
No inhibition	9	45	1	4

sympathetic nerves. These sensitized the cardio-vagal mechanism in the following order of decreasing effectiveness as compared to the heart extract:

Heart-liver-brain-skeletal muscle-ovaries-lungs (figs. 1 and 2). Ten males and 10 females were used and in all cases the above mentioned phenomenon was demonstrated.

As a control experiment, five extracts of different vagus refractory hearts were prepared. These extracts gave no sensitizing effects when applied to other vagus refractory hearts.

Since the results obtained above on the toads and frogs indicated a relatively higher percentage of frogs which respond by cardiac inhibition on stimulation of the vago-sympathetic nerves among the females, it was logical to test out the effects of more highly purified ovarian extracts.

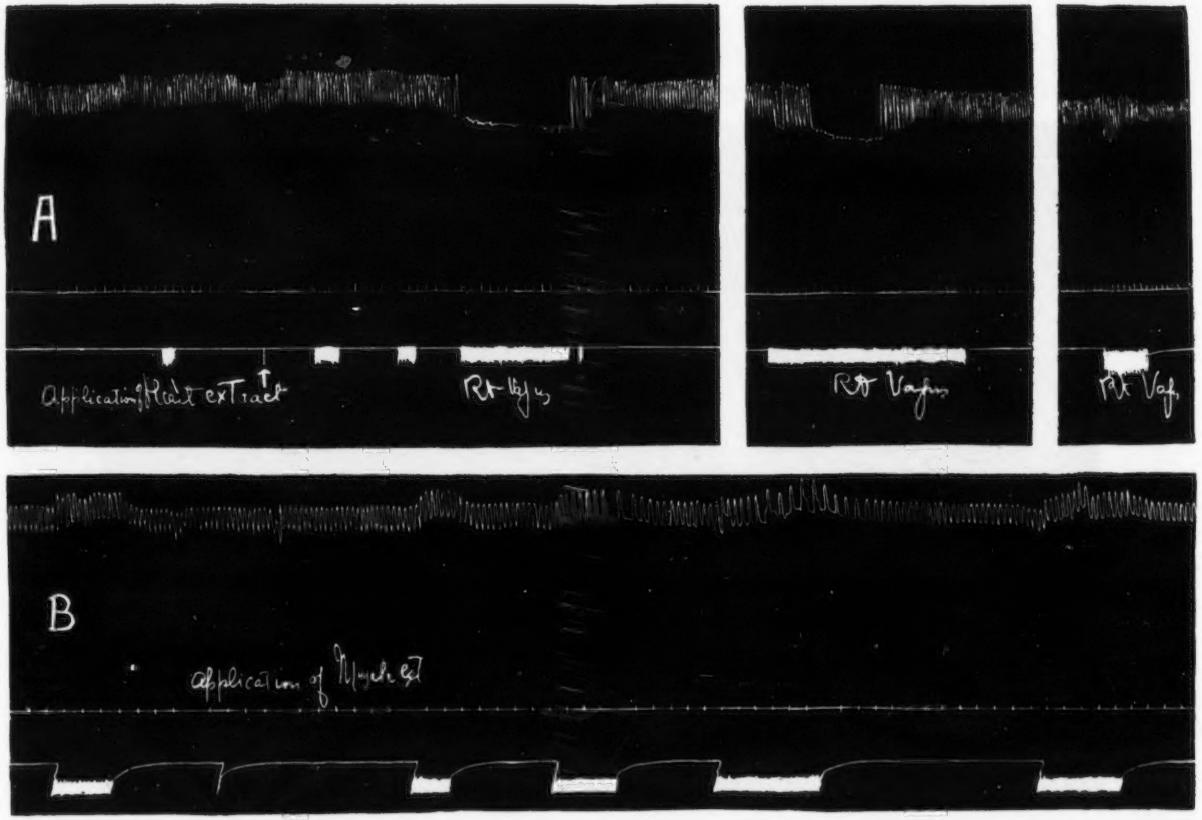

Fig. 1. Showing the effects of (A) the heart extract and (B) the skeletal muscle extract on the sensitivity of the vago-cardiac mechanism of vago-refractory hearts.

Note the temporary appearance of cardiac inhibition after the application of the extract.

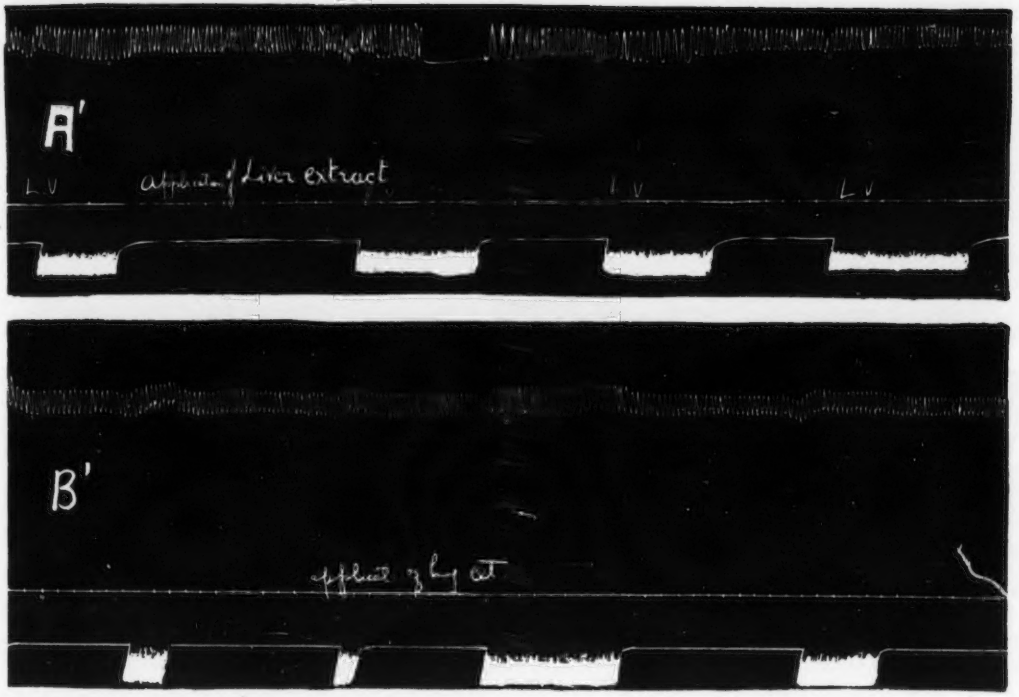

Fig. 2. Showing the effects of (A′) the liver extract and (B′) the lung extract on the sensitivity of the vago-cardiac mechanism of vago-refractory hearts.

The ovarian extract, as prepared by Gustavson (18), dissolved in Ringer solution, was applied to the vago-refractory frog hearts. Instead of an increased sensitivity in the vagus refractory hearts there appeared a permanent slowing of the heart beats and no increase in the influence of the vagi on the cardio-inhibitory mechanism. When the ovarian extract was applied to the hearts which give rise to cardiac inhibition on vago-sympathetic stimulation, the inhibitory effects disappeared for at least one hour and no further inhibition could be obtained with activation with heart extracts of vago-reactive hearts. An exactly similar phenomenon was obtained when the male hormone preparation, as prepared by Gallagher (19), was used. The ovarian extract was tested on four frogs and the male hormone on two. These effects may be due to products other than the sex hormones present in the extracts.

DISCUSSION. Sex differences in other reactions in frogs have been described at some length by Burridge (29). He reports that during the winter season male frogs recover from spinal shock much earlier than the female frogs. The aorta of the male frog is brittle as compared to the aorta of the female. An increased KCl content of the perfusing Ringer's solution brings about a cardiac arrest in the female heart while the male heart exhibits a strong contracture. Burridge attributes all these effects to the possible higher calcium content in the tissues of the male frogs.

The inconstancy of the results, showing that a good many male frogs exhibit a distinct cardiac inhibition on stimulation of the vago-sympathetic nerves, may find a possible explanation in the histological description of Tso-Hsin Cheng (21). Cheng found in normal male frogs ovarian elements (or oöcysts) scattered among the testicular tissues. Such an occurrence might give rise to variations which should be followed up by further histological examinations of the presence of a relationship between the cardiac inhibition discussed and sex. The results of this work indicate the presence in the heart and some of the other organs of a substance or substances that sensitize the vago-cardiac mechanism. The heart and the other organs of frogs which do not show cardiac inhibition on stimulation of the vago-sympathetic nerves seem to be lacking this substance, since the addition of an extract from these organs fails to sensitize vago-refractory hearts. It is impossible to decide, from the present data, whether the ovarian hormone is in some way related to the vago-cardiac sensitizing substance because our crude ovarian extract of the frog showed a less effect than some other tissues. On the other hand, the highly concentrated ovarian extract of Gustavson showed no sensitizing effects.

The highest concentration of the substance seems to exist in the heart tissue itself and the concentration is decreasing in the other tissues as described and illustrated in the results.

The substance is apparently different from Loewi's (22) vagal substance,

since the latter is in the perfusate and on extracting the heart tissue it is destroyed while the former is present in the extract. However, the physiological effects ascribed to both substances have some resemblance, since both are concerned with vagal inhibition. The substance described in this paper only sensitizes the vago-cardiac mechanism, while Loewi's substance produces an inhibition without any vagus stimulation.

I wish to thank Doctor Carlson for his constant guidance in carrying out this work.

SUMMARY

1. Sex differences have been observed in the response of the heart of toads and frogs to stimulation of the vago-sympathetic nerves. More marked and consistent cardiac inhibition was noted in the females.

2. A vago-cardiac sensitizing influence from crude organ extracts is described. This substance (or substances) is present in highest concentration in the extracts of vago-responsive hearts and at lower concentrations in the tissues of frogs which respond by cardiac inhibition to vagal stimulation. The application of such extracts to vago-refractory hearts, renders them vagus responsive for a short time (1 to 30 minutes).

3. Similar extracts of the heart and other organs of vago-cardiac refractory frogs do not sensitize the frog's vago-cardiac mechanism.

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THE DIFFERENCES IN ELECTRICAL RESPONSE OF THE RETINA OF THE FROG AND HORNED TOAD ACCORDING TO THE POSITION OF THE ELECTRODES

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Received for publication June 23, 1930

The work reported in this paper is a continuation of the study of the electrical responses of the retina begun in 1920. The experiments here presented were directed to the study of such differences as might exist in the electrical responses from various positions on the retina of the frog's eye and of the horned toad's eye.

The apparatus used in this study is essentially the same as that used in the former work and is described in detail in the first paper.[1] Briefly it consists of the following elements: a moist eye-chamber containing the eye and connecting electrodes; a two-stage resistance-coupled vacuum-tube amplifier; and a Hindle string galvanometer with photographic recording attachment.

The retina is exposed to light of known intensity and duration and the potential changes in the retina resulting from this stimulating light are conducted to the amplifier through electrodes described later. After amplification, the potential changes are traced by the image of the galvanometer string on a revolving strip of bromide paper. A secondary galvanometer records on the same paper the exact moments the stimulating light falls and ceases to fall upon the eye.

The eye, in this work as in the former work referred to above, was cut in half, thus removing the lens and iris. The posterior half with exposed retina was placed in the eye-chamber on a central insulating pedestal. Formerly zinc-sulphate electrodes were used to connect from the eye to the

[1] "The Electrical Response of the Retina under Stimulation by Light." Chaffee, Bovie and Hampson. Journ. Optical Soc., January, 1923, vii, no. 1.

Other papers dealing with the same experiment are: "Effects of Varying the Wave Length of the Stimulating Light upon the Electrical Response of the Retina." E. L. Chaffee and Alice Hampson. Journ. Optical Soc. July, 1924, ix, no. 1; "Electrical Response of the Retina in Different Types of Cold-Blooded Animals." A. B. Meservey and E. L. Chaffee. Journ. Optical Soc., December, 1927, xv, no. 6; "Normal and "Rodless" Retinae of the House Mouse with Respect to the Electromotive Force Generated through Stimulation by Light." Keeler, Sutcliffe and Chaffee. Proc. National Academy of Sciences. June, 1928, xiv, no. 6.

amplifier. Each of these electrodes consisted of an amalgamated-zinc wire dipping in zinc-sulphate solution and a thread moistened with Ringer's solution extending from the zinc-sulphate solution to the eye. Because of the slight possibility that zinc ions might diffuse along the threads to the eye, calomel electrodes were substituted for the zinc electrodes in this work. It should be remarked, however, that in the former work no positive evidence that zinc reached the eye was obtained.

Each calomel electrode possessed a connected external tube filled with potassium chloride which dipped into one arm of a separate U-tube. This U-tube had an agar plug at its lowest point which separated the potassium chloride solution in the side communicating with the calomel electrodes from the Ringer's solution on the other side. A thread moistened with Ringer's solution extended from the Ringer's solution in the U-tube to the eye.

The connections to the retina were made in essentially the same way as in the former work. The thread electrode which made connection through one calomel electrode with the grid of the amplifier was stood on end in the cup of the eye, thus making contact with the retina at a single small point. Tests show that the potential changes recorded by the apparatus are those produced in the portions of the retina immediately surrounding the point of contact of this electrode. The other electrode made connection between the outside or sclerotic coat of the eye and the grounded filaments of the amplifier. This electrode is called the grounded electrode.

In any one experiment the intensity of the stimulating light and the time of exposure were kept constant. Exposures of two or five seconds' duration were usually used. All eyes were used at room temperature, which was about 23°C. Sufficient time for dark adaptation was allowed between all exposures. Approximately even illumination of the whole retina was used throughout this series of experiments.

1. Experiments using the frog's eye. When subjecting the retina of the frog to uniform illumination of two seconds' exposure, at least one minute and usually more was allowed between exposures for dark adaptation.

First, tests were made to determine differences, if any, in the responses according to the position and shape of the electrode which made contact with the sclerotic coat. A small point of contact, as with a moist thread touching end on, gives responses showing slightly more fine structure than are obtained using a large pad electrode. A very great increase in the amount of fine structure in the response when the contacting electrode on the retina is small has already been recorded, and hence, the retina electrode is always a thread touching end on. A test made some years ago bears on this point. It was shown that the magnitude of the response increased to a maximum and then rapidly decreased as a point of illumination on the retina was caused to approach and pass the point of contact

of the thread electrode on the retina. The deduction is then that the small electrode taps the potential of a relatively small number of end organs immediately surrounding the electrode and hence, it is reasonable to expect that the recorded potential changes are less smoothed out.

With the high resistance sclerotic coat separating the active elements of the retina and the outside electrode, less improvement is to be expected as observed in the use of a small electrode on the sclerotic coat. The use of a small electrode on the outside of the eye has the disadvantages that little Ringer's solution is in contact with the eye and drying and dying are hastened. A doughnut-shaped electrode, on which the sclerotic coat rested, was used as a compromise between the more ideal fine contact and the large pad.

When the position of the outside electrode was varied there was no detectable change in the size or shape of the response. This observation indicates that the sclerotic coat is practically an equipotential surface, and has the same potential as that portion of the optic nerve which protrudes through the coating.

Some differences were observed in the size and shape of the electrical response when the position of the thread electrode contacting with the retinal surface was varied. These differences were greater for high intensities of illumination of the retina. Accordingly, in most of these tests with the retina of the frog the intensity of light at the retina was greater than 0.1 meter candles.

Figure 1 shows the main characteristics of the typical electrical response from the retina of the frog for a time exposure of fairly high intensity. It consists of a latent period, T_L; a small negative deflection often called the A deflection; a large positive rise called the B rise, which gradually declines from its maximum height; and a much slower second positive rise known as the C rise. The C rise occurs only at high intensities, *i.e.*, intensities greater than 0.1 meter candle, and its maximum may be many seconds after this stimulating light ceases to act, as shown in figure 1. The "off" effect is another small positive rise superimposed on the curve after a brief latent period.

In the first paper it was shown that if the intensity of stimulating light is small, *i.e.*, of the order of 250 micrometer candles at the retina, there are two maxima discernible on the B rise, one reaching its maximum in about 0.3 second and the second attaining a maximum in about 0.7 second. These maxima are called B_1 and B_2 respectively. It was suggested that the first maximum is due to cones and the second to rods. As the intensity of illumination is increased the first maximum increases in height more rapidly than the second maximum so that when the intensity is much above 300 micrometer candles, the first maximum is so large as to mask the second.

The high intensity of illumination used in these experiments precluded more than a suggestion of the B_2 maximum described above and attributed to the rods. Some response curves have a suggestion of the B_2 maximum as a break in the decline from the main maximum of the B rise.

On the other hand, at these high intensities the B_1 rise is much broader than at low intensities. The electrical potential rises rapidly at first to a value after which the increase is more gradual up to the maximum of response. The potential then declines at first rapidly and then more slowly. This structure suggests that the B_1 response may be made up of two (or perhaps more) components.

The structure described above is illustrated by the first response curve of plate I, which is quite typical of the response of the frog's retina at high intensity (0.455 meter candles).

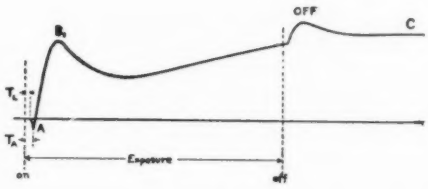

Fig. 1

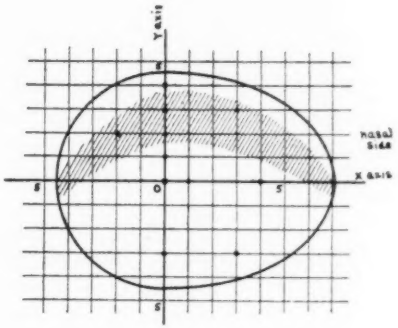

Fig. 2

Fig. 1. Typical response of the retina of the frog for high intensity of illumination. Fine structure omitted.

Fig. 2. Retina of the frog. Shaded area is area centralis.

Many responses have been recorded when the grid electrode was placed at various positions on the retinal surface. These positions were identified in terms of the x and y coördinates as illustrated in figure 2. The most marked difference in the responses is the variation of height with position. In general, the response is small at the edges of the retina and at the blind spot which is taken as the origin of coördinates in figure 2. The response is usually greater from the upper portion of the retina than from the portion below the blind spot, and in general the response is greater from the shaded area of figure 2 which represents the *area centralis*.

Besides the variation in magnitude of the responses from various positions on the retina there is a marked difference in structure or shape. The curve shown in plate I (a) is from point x = 1, y = 0 (the unit being approximately one-tenth the horizontal dimension of the eye) and in shape is

typical of the response from points outside the area centralis. Curve (b) is similar and is from point x = 0, y = −3. Curve (c), plate I, is for point x = 0 and y = 3 in the area centralis. This response shows marked oscillation on the maximum and also on the "off" effect. The responses from the *area centralis* usually show these oscillations and the maximum is usually broader than for the responses from points outside the *area centralis*. This latter observation is confirmed by the fact that the measured time from the beginning of exposure to the highest point is greater for the responses from the *area centralis*.

The time interval T_L (see fig. 1) was found to be essentially constant for all positions of the inside electrode. The average value of T_L was found to be about 0.045 second.

The measurements of the time interval $T_A - T_L$, although subject to considerable experimental error, suggest that this time interval may be slightly greater for those curves obtained from the *area centralis*. This increase would be consistent with the increased time to the maximum of the *B* rise for the points in the *area centralis*. The average value of $T_A - T_L$ for points outside the *area centralis* is about 0.037 second. These values of the time intervals referred to above are for constant intensity of light at the retina of 0.455 meter candles. Of course the time T_L and $T_A - T_L$ vary with intensity.

Reference has already been made to the oscillations that occur on the top of the *B* rise and often on the "off effect," and to the fact that these oscillations are much more prominent when the grid electrode is situated in the *area centralis*. The obvious explanation of these oscillations is that they are the resultant of many nerve impulses coming either from the end organs, the rods and cones, or from the fibres which spread over the inside surface of the retina and extend from the end organs to the exit of the optic nerve.

The major rises in potential such as the *B* rise may be due also to the summation of the many nerve responses, the small oscillations being the unsmoothed sum of these nerve impulses due to the finite number of impulses contributing to the observed potential or to a certain possible coupling between the nerves causing the nerves to discharge more or less in step. Or again, the *B* rise and the other major changes in potential may be due to a separate effect secondary to and dependent upon the nerve responses. To investigate further this question both electrodes to the amplifier were made to touch the inside of the retina at small points. The ground electrode (connected to filament of amplifier) was made to touch the blind spot which is the exit of the optic nerve and the point of convergence of all the nerve fibres of the retina. The grid electrode was placed at various positions on the retina. Curves (d), (e), (f), (g), and (h), plate I, are typical of the results. The data for these curves are given on the page opposite plate I.

Photographs (g) and (h), plate I, show almost a total absence of the *B* rise although the oscillations are present. Curve (h) is for an intensity of light less than one-thousandth that for curve (g). It is to be noted that

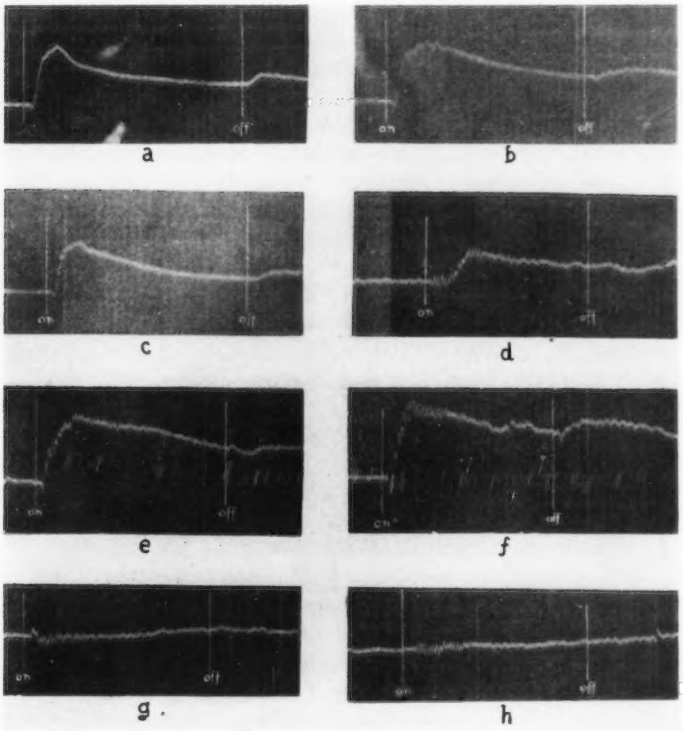

Plate I. Responses of retina of the frog. Time of exposure, 2 seconds. Temperature (approximate), 23°C.

	PHOTOGRAPH							
	a	b	c	d	e	f	g	h
Intensity (meter candles)	0.455	0.455	0.455	0.00084	0.86	0.86	0.86	0.00084
Position of grid electrode	1.0	0.3	0.-3	0.2	0.2	0.-2	0.1	0.1
Position of ground electrode	Outside	Outside	Outside	0.0	0.0	0.0	0.0	0.0

the frequency of oscillation does not vary appreciably with intensity of light.

Photograph (d), plate I, is for low intensity of illumination and shows a cancellation of the first part of the *B* rise but a small portion of the last

part of the *B* rise is recorded. Curves (e) and (f) show strong oscillation with considerable of the major *B* rise.

Many experiments have been made in which both electrodes were placed at various points on the retina. All experiments indicate that the main positive rises in the curves of response, *i.e.*, the *B* and *C* rises, are due to potentials developed across the retinal layer from the sclerotic coat to the nerve layer on the inner surface, and have their seat in some or all of the several layers in the retina. The fine oscillations are due to potentials which are not cancelled by placing both terminals on the retina, as are the main potential changes, and hence must be directed partly at least along the surface of the retina, as would be true if they were due to action currents in the nerves which spread over the inner surface of the retina. This matter of oscillations will be described further in a later paper.

To summarize, no two points on the frog's retina give identical responses with respect to the sclerotic coat, but the variations over the retina are gradual. The greatest divergences from the typical response are found in the *area centralis*. Accordingly, the differences in response are probably due to differences in the relative numbers and kinds of rods and cones at the various points investigated. The main positive rises in the curves of response are due to potentials which develop across the retinal layer. The fine oscillations are probably due to nerve responses, and are produced by potentials which are directed in part at least along the surface of the retina.

2. Experiments using the retina of the horned toad. The experiments using the eye of the horned toad are similar to those described above for the frog. The horned toad was chosen principally because its retina contains only cones while the frog's retina has both rods and cones.

At the present time, the investigation on the horned toad's retina has not progressed as far as that with the frog's. The results presented here are mostly qualitative. Time relations of the various maxima and minima were not measured because the responses are very quick in comparison with those of the frog's retina, and the apparatus is not suitable for accurately measuring such short time intervals.

The heights of the various responses from different points of the retina are only approximate for the following reasons. Two factors, practically negligible in the frog's eye, enter to affect the heights of the responses in the horned toad's eye. The first is that the response depends greatly upon the amount of dark or light adaptation of the eye. For the retina of the frog, five minutes in the dark is sufficient for dark adaptation. The horned toad's eye seems to require a much longer time to come back to its original condition. Secondly, the horned toad's eye dies more rapidly than the frog's eye under the rough treatment of moving the electrode around on the retina, though it shows no change in response during six

hours if left undisturbed. The changes in response due to dying have been eliminated by choosing the positions of the grid electrode on the retina in haphazard order and by experimenting upon a very large number of eyes. A response was considered typical of a particular region of the retina only when it retained its characteristic form through series of tests for six hours after excision of the eye.

All the conditions under which the responses were measured were the same as for the frog's eye, except for the intensity of the stimulating light. Much higher intensities (33 to 133 meter candles) were used with the horned toad's eye because of the immensely higher threshold of the horned toad's retina.

Figure 3 shows the main characteristics of the typical response from a certain region of the retina of the horned toad when the stimulating light has a relatively high intensity. The time of exposure represented in figures 1 and 3 is about $2\frac{1}{2}$ seconds.

The most marked differences in the general characteristics of the responses from the retina of the frog and horned toad, beside the much greater intensity of light required for the horned toad, are: 1, shorter latent time T_L and times to the maxima of the A and B rises in the responses from the horned toad's eye; 2, much more pronounced and quicker "off effect" from the retina of the horned toad; 3, the deflection after the B_1 rise sinks to lower levels and is often negative in the case of the responses from the horned toad; 4, entire absence of C rise in the response curves from the horned toad's retina.

Since the times T_L, T_A and time to the maximum of B_1 for the responses from the frog's retina become less as the intensity of light is increased, the smaller values of these time intervals observed in the responses of the horned toad's eye are consistent with the higher intensities of stimulating light necessary if, as is probably true at high intensities, these times and the B_1 rise of the response from the frog's eye are determined largely by the cone response. The absence of the C rise and the lower level of potential after the B_1 rise of the horned toad curve can be attributed to the absence of rods in the retina of the horned toad.

Tests in which the position of the ground electrode on the sclerotic coat was varied showed that the sclerotic coat of the horned toad's eye is, as in the case of the frog's eye, an approximately equipotential surface. There was found, however, somewhat more variation of potential than in the experiments with the frog due probably to the fact that the sclerotic coat of the horned toad's eye is much thinner than that of the frog's eye. In all further work reported in this paper contact was made through a pad moistened with Ringer's solution on which the eye rested.

Experiments were then made in which the position of the thread grid electrode on the inside of the retina was varied. In choosing positions for

the grid electrode on the horned toad's retina no fixed series of points was used each time, but the positions were chosen at random and in haphazard order, as explained above.

Figure 4 is a diagram of the right eye of the horned toad. A system of coördinates has been arranged on the retina to locate the positions of the grid electrode. The origin of coördinates is the blind spot and entrance of the pecten. At about (0, 2) is the fovea of the eye. The distance between parallel lines in figure 7 is about 0.75 mm.

After a very few experiments, it appeared that some points of the retina always gave negative responses, while others gave the positive responses of the type shown in figure 3. The region which gave negative responses was finally definitely located as an almost horizontal band across the retina corresponding to the shaded area in figure 4. Below and above this band, the responses were positive. On the borderlines between the regions pe-

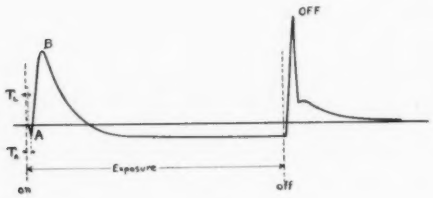

Fig. 3

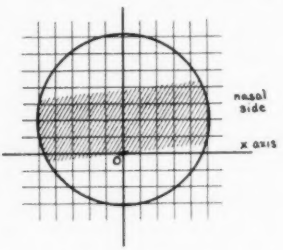

Fig. 4

Fig. 3. Typical positive response of the retina of the horned toad.

Fig. 4. Retina of the horned toad. Shaded area is region giving negative responses.

culiar responses, apparently a mixture of positive and negative potentials, were obtained. For the left eye, the negative band was the mirror image of that in the right.

The typical negative response from the shaded area of figure 4 is similar to the typical positive response of figure 3 except of course inverted. There is one difference, however, which is practically always present, namely, the flat region after the B rise is negative in both types of response.

Photograph (a), plate II, is a typical positive response from point x = 4, y = 0 on the coördinate system shown in figure 4. It was taken with an illumination on the retina of 33. meter candles. The maximum *B* rise in (a) corresponds to about 1 millivolt. It is rather peculiar that so large a response is obtained at a point near the border line between the two regions, but experience has shown that some of the largest positive responses are obtained from this particular region. Photographs (b), (c) and (d) are negative responses from points (3, 2), (1, 2) and (−2, 2), respectively.

The light intensity was in all three cases 133 meter candles. The "off" effect is purely negative only near the fovea, which is at about point (0, 2). The first positive deflection is also largest near the fovea.

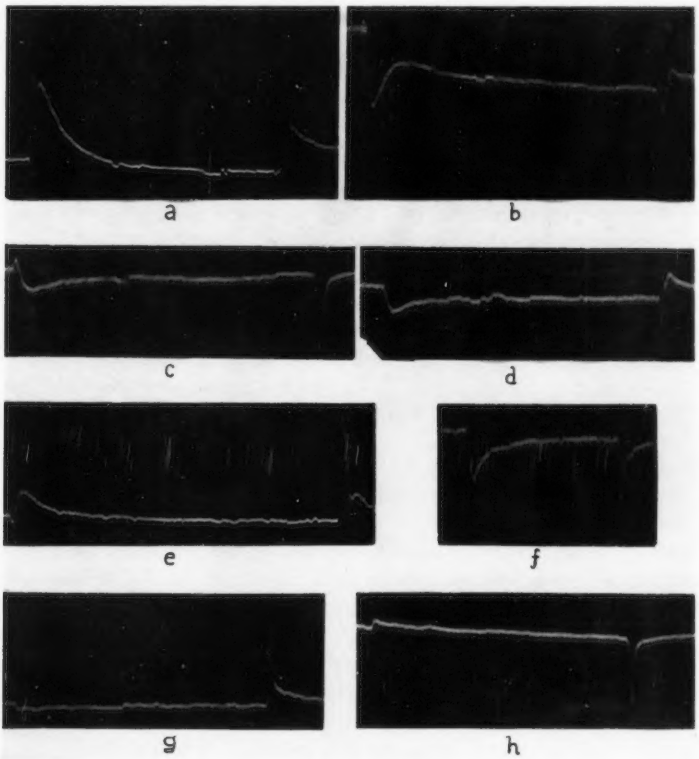

Plate II. Responses of retina of horned toad. Time of exposure, 5 seconds. Temperature (approximate), 23°C.

	PHOTOGRAPH							
	a	b	c	d	e	f	g	h
Intensity (meter candles)	33.3	133	133	133	133	133	133	133
Position of grid electrode	4, 0	3, 2	1, 2	−2, 2	0, −2	0, 2	4, 0	0, 2
Position of ground electrode	Sclerotic coat	Sclerotic coat	Sclerotic coat	Sclerotic coat	Sclerotic coat	3, −1	Sclerotic coat	Sclerotic coat

In the positive area below the blind spot the typical response is similar in form to the curve of figure 3, as described above. Photograph (e), plate

II, is a positive response from the point $(0, -2)$. The intensity of illumination was 133 meter candles.

The upper positive band of the eye is not the same as the lower positive band. Responses were very difficult to obtain when the grid electrode was placed in this upper region. When the eye was fresh, a response like that in photograph (f), plate II, usually was obtained. It consisted of an "on" effect similar to that at the point $(0, -2)$ (photograph e), but the "off" effect was usually entirely missing or at the most a small diphasic jerk.

As the eye aged, this upper positive band usually ceased giving responses before other parts of the retina. The negative responses died out next, while the positive responses from below the blind spot persisted longest. "Off" effects usually persisted longer than "on" effects.

Some experiments were made as with the frog's eye, by placing both electrodes on the retina. When one electrode is placed in the negative response band and the other in the region giving positive responses, a large response would be expected as shown in photograph (f), plate II. The grid electrode was at point (0, 2) and the ground electrode at $(3, -1)$. This response is larger than was obtained with either electrode on the outside pad.

It has not been possible to demonstrate marked oscillations by placing the two electrodes very close together on the retina of the horned toad as was done with the frog's eye.

Oscillations are, however, sometimes found in the responses from the eye of the horned toad. They appear quite regularly on the *B* rise of the positive responses (photograph (g), plate II), but have not been observed on responses from the negative band. They are also found to be quite marked in the border line regions between positive and negative bands.

So far, abnormal responses have not been considered. But since as the eye dies the several parts of the response decrease at different rates, a response of an abnormal or dying eye is easily identified. Photograph (g) shows an abnormal response from the point (4, 0) in the lower positive band. Photograph (h) is also an abnormal response from the point (0, 2) in the negative band. In every case, then, the first effect of dying is the disappearance of the *B* rise of the "on" effect. After this, the remainder of the response simply becomes smaller and finally disappears altogether. In no case has a dying positive response resembled a normal negative response or vice-versa.

To summarize, the electrical responses from the horned toad's retina are either positive or negative, depending upon the position of the grid electrode. The negative responses are obtained when the grid electrode makes contact with the retina in a band which extends across the eye just above the pecten and blind spot. The negative responses are similar to

the positive responses inverted except that the flat region between "on" and "off" effects is always negative. Responses, part positive and part negative, can be obtained on the borders between regions. Oscillations are present on the "on" effects from the positive regions but are less marked than in the responses from the frog's retina, probably because they are more rapid and less easily resolved.

The authors express their appreciation for the assistance in this work afforded by grants from the Milton and Rumford funds.